

Olerup SSP® HLA-B*35

Product number:	101.522-24/03 – including <i>Taq</i> pol.
Lot number:	07L
Expiry date:	2013-September-01
Number of tests:	24 tests – Product No. 101.522-24 3 tests – Product No. 101.522-03
Number of wells per test:	72
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

This Product Description is only valid for Lot No. 07L.

**CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*®
HLA-B*35 LOT**

The HLA-B*35 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP*® HLA-B*35 lot was made (Lot No. 58G).

Twenty-four wells have been added to the HLA-B*35 kit,
wells **49 to 72**.
The amplification patterns for some rare HLA-B*35 alleles only differ
by the length of the specific PCR products.

The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot.

Well	5'-primer	3'-primer	rationale
5	Exchanged	-	Improved allelic resolution.
25	-	Added	Increased yield of specific PCR product.
31	Added	-	Increased yield of specific PCR product.
38	Moved	Moved	Primer pair moved to well 72 for improved specificity.
46	Moved	Moved	Primer pair moved to well 49 for decreased primer oligomer formation.
49	Added	Added	Primer pair from well 46.
50	New	New	New primer pairs for the B*35:101:01 and B*35:101:02 alleles.
51	New	New	New primer pairs for the B*35:100, B*35:102 and B*35:145N alleles.

52	New	New	New primer pairs for the B*35:104 and B*35:141 alleles
53	New	New	New primer pairs for the B*35:103 and B*35:129N alleles.
54	New	New	New primer pairs for the B*35:106 and B*35:110 alleles.
55	New	New	New primer pairs for the B*35:108:01 and B*35:108:02 alleles.
56	New	New	New primer pairs for the B*35:107 and B*35:128 alleles.
57	New	New	New primer pairs for the B*35:111 and B*35:149 alleles.
58	New	New	New primer pairs for the B*35:112, B*35:115 and B*35:139 alleles.
59	New	New	New primer pairs for the B*35:116 and B*35:127 alleles.
60	New	New	New primer pairs for the B*35:120 and B*35:121 alleles.
61	New	New	New primer pairs for the B*35:122, B*35:123 and B*35:139 alleles.
62	New	New	New primer pairs for the B*35:132 and B*35:144 alleles.
63	New	New	New primer pair for the B*35:143 allele.
64	New	New	New primer pairs for the B*35:130N and B*35:134N alleles.
65	New	New	New primer pairs for the B*35:131 and B*35:148 alleles.
66	New	New	New primer pairs for the B*35:105, B*35:113 and B*35:133 alleles.
67	New	New	New primer pair for the B*35:138 allele.
68	New	New	New primer pairs for the B*35:137 and B*35:140 alleles.
69	New	New	New primer pairs for the B*35:143 and B*35:147 alleles.
70	New	New	New primer pair for the B*35:117 allele.
71	New	New	New primer pairs for the B*35:119 and B*35:126 alleles.
72	Added	Added	Primer pair from well 38.

Changes in revision R01 compared to R00:

1. Primer mix 38 contains the 800 bp positive control primer pair. This has been corrected in the specificity and interpretation tables.

Change in revision R02 compared to R01:

1. The HLA-B*27:06, 27:21, 44:05:01-44:05:02, 44:25, 44:42, 44:58N, 44:70, 44:75, 44:78 and 54:15 and the C*01:32 alleles are weakly amplified by primer mix 6. This has been changed in the Specificity and Interpretation Tables.

Change in revision R03 compared to R02:

1. A typing mistake on page 1 has been corrected.

Change in revision R03 compared to R02:

1. Primer mix 26 does not amplify the B*35:42:02 allele. This has been corrected in the specificity and interpretation tables. Thus, this lot of the HLA-B*35 kit does not separate the B*35:01:01:01-35:01:03, 35:01:05-35:01:16 and 35:01:18-35:01:22 and the B*35:42:02 alleles.

Change in revision R05 compared to R04:

1. Primer mix 46 does not amplify the B*35:96 and B*35:98 alleles. This has been corrected in the specificity and interpretation tables. Thus, this lot of the B*35 subtyping kit cannot distinguish the B*35:98 and the B*35:03:01 and 35:03:03-35:03:06 alleles.

PRODUCT DESCRIPTION

HLA-B*35 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*35:01 to B*35:151 alleles.

PLATE LAYOUT

Each test consists of 72 PCR reactions in a 96 well cut PCR plate. Wells 73 to 96 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32
33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48
49	50	51	52	53	54	55	56
57	58	59	60	61	62	63	64
65	66	67	68	69	70	71	72
empty	empty	empty	empty	empty	empty	empty	empty
empty	empty	empty	empty	empty	empty	empty	empty
empty	empty	empty	empty	empty	empty	empty	empty

The 96 well cut PCR plate is marked with 'HLA-B*35' in silver/gray ink.

Well No. 1 is marked with the Lot No. '07L'.

A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.

The PCR plates are covered with a PCR-compatible foil.

INTERPRETATION

Due to the lack of B*35-specific sequence motifs, the interpretation of HLA-B*35 SSP subtypings will be influenced by many other HLA-B alleles. In addition, the A*01:41 allele will be weakly amplified by primer mix 11. Furthermore, the A*68:56 and the C*06:20 and C*12:50 alleles will be amplified by primer mix 39, the C*01:10, C*02:02:14 and C*07:15 alleles will be weakly amplified by primer mix 30, the C*01:32 allele is weakly amplified by primer mix 6, the C*02:03, C*04:34, C*05:12, C*07:95 and C*08:39 alleles will be amplified by primer mix 23, the C*03:05, C*03:25 and C*03:27 alleles will be amplified by primer mix 28, the C*03:28, C*03:49, C*04:80, C*05:10 and C*15:08 alleles will be amplified by primer mix 11, the C*03:90 allele will be amplified by primer mix 34, the C*03:102 allele will be amplified by primer mix 2, the C*05:30 allele will be amplified by primer mixes 58 and 61, the C*06:21 allele will be amplified by primer mix 63, the C*07:04:01-07:04:04, C*07:11-07:12, C*07:45, C*07:101, C*07:139 and 07:142 alleles will be weakly amplified by primer mix 47, the C*07:63 allele will be amplified by primer mixes 10 and weakly by primer mix 47, the C*07:123 allele will

be amplified by primer mixes 3 and 18, the C*15:02:05 and C*15:06:03 alleles will be amplified by primer mix 3, the C*15:09, C*15:19-15:20, C*15:27 and C*16:20 alleles will be amplified by primer mix 18 and the C*15:39 allele will be amplified by primer mix 22.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*35 alleles, i.e. **B*35:01 to B*35:151**, recognized by the HLA Nomenclature Committee in October 2010¹ will give rise to unique amplification patterns by the primers in the HLA-B*35 subtyping kit².

The B*35:122 and B*35:123 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 61.

The B*35:132 and B*35:144 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 62.

The B*35:130N and B*35:134N alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 64.

The B*35:131 and B*35:148 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 65.

The HLA-B*35 subtyping kit cannot distinguish the B*35:01:01:01-35:01:03, 35:01:05-35:01:16 and 35:01:18-35:01:22 alleles, the B*35:02:01-35:02:04 alleles, the B*35:03:01 and 35:03:03-35:03:06 alleles, the B*35:04:01-35:04:02 alleles, the B*35:05:01-35:05:02 alleles, the B*35:08:01-35:08:02 and 35:08:04 alleles, the B*35:12:01-35:12:02 alleles, the B*35:20:01-35:20:02 alleles, the B*35:24:01-35:24:02 alleles, the B*35:42:01-35:42:02 alleles, the B*35:43:01-35:43:02 alleles, the B*35:101:01-35:101:02 alleles or the B*35:108:01-35:108:02 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2010-October-15, release 3.2.0, www.ebi.ac.uk/imgt/hla.

²This lot of the B*35 subtyping kit cannot distinguish the B*35:98 and the B*35:03:01 and 35:03:03-35:03:06 alleles.

SPECIFICITY TABLE

HLA-B*35 SSP subtyping

Specificities and sizes of the PCR products of the 72 primer mixes used for HLA-B*35 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*35 alleles ^{3,4}	Other amplified Class I alleles ⁵
1⁶	95 bp	800 bp	*35:01:01:01-35:09:02, 35:11:01-35:12:02, 35:14:01-35:15, 35:17-35:18, 35:20:01-35:24:02, 35:27, 35:29-35:45, 35:48, 35:50-35:62, 35:64-35:68:02, 35:70-35:72, 35:74-35:75, 35:77-35:79, 35:81-35:151	*08:32, 18:01:01-18:11, 18:13-18:15, 18:17N-18:36, 18:38-18:47, 18:49-18:52, 37:08, 38:06-38:07, 39:19:01-39:19:02, 44:06, 51:01:01-51:01:10, 51:01:12-51:24:04, 51:26-51:46, 51:48-51:103, 51:105-51:106, 53:01:01-53:16, 53:18-53:24, 78:01-78:04, 78:07
2	150 bp	1070 bp	*35:01:01:01-35:04:03, 35:06-35:08:04, 35:10-35:17, 35:19-35:21, 35:23-35:30, 35:33-35:36, 35:38-35:42:02, 35:45-35:50, 35:52, 35:54-35:57, 35:59, 35:61-35:63, 35:65Q, 35:69-35:71, 35:74, 35:76-35:78, 35:80-35:85, 35:90-35:96, 35:98, 35:100-35:101:02, 35:103-35:113, 35:115-35:116, 35:120-35:126, 35:128-35:134N, 35:136-35:150	*08:49, 13:01:01-13:01:05, 13:06-13:07N, 13:12-13:13, 13:17, 13:20, 13:22:01-13:23, 13:25-13:26, 13:28-13:29, 13:36, 13:39, 14:10, 15:02:01-15:02:05, 15:13, 15:20-15:21, 15:25:01-15:25:03, 15:36, 15:44, 15:62, 15:77, 15:80, 15:85, 15:88-15:89, 15:106, 15:112, 15:121, 15:139, 15:144, 15:154, 15:165, 15:170, 15:194, 15:204, 18:22, 27:19, 27:30, 37:01:01-37:01:07, 37:03N-37:06, 37:08, 37:10-37:11, 37:13-37:25, 38:20, 39:42, 40:28, 44:02:01:01-44:14, 44:16-44:17, 44:19N, 44:21-44:30, 44:32-44:40, 44:42-44:46, 44:48-44:52N, 44:55-44:64:02, 44:66-44:98, 44:101-44:105, 44:107-44:115, 48:02:01-48:02:02, 51:04, 51:42, 51:46, 51:56, 53:01:01-53:13, 53:15-53:24, 55:14, 56:09, 56:11-56:12, 57:01:01-57:01:04, 57:01:06-57:10, 57:12, 57:14-57:20, 57:22-57:30, 57:32-57:37, 58:01:01-58:01:02, 58:01:04-58:01:08, 58:04-58:05, 58:09-58:15, 58:17N, 58:19, 58:21-58:24, 58:28-58:31N, 83:01, C*03:102

Lot No.: **07L**

Lot-specific Information

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3⁶	115 bp	800 bp	*35:01:01:01- 35:01:22, 35:03:01, 35:03:03-35:03:07, 35:05:01-35:05:02, 35:07-35:08:04, 35:10-35:11:01, 35:13-35:14:01, 35:15-35:17, 35:19- 35:21, 35:23-35:30, 35:32-35:42:02, 35:45-35:58, 35:61- 35:63, 35:65Q- 35:66, 35:68:01- 35:72, 35:74-35:78, 35:80, 35:82, 35:84- 35:85, 35:89-35:94, 35:96-35:98, 35:100-35:101:02, 35:103-35:113, 35:115-35:116, 35:119-35:120, 35:122-35:128, 35:130N-35:134N, 35:136-35:148, 35:150-35:151	*07:02:13, 07:22:02, 07:68:02, 08:07, 08:37, 13:26, 13:35, 15:03:02, 15:05:02, 15:20, 15:27:03, 15:38:02, 15:39:02, 15:179, 18:01:01-18:15, 18:17N-18:19, 18:22-18:25, 18:27-18:52, 37:09, 39:07, 39:15, 39:29, 39:43, 39:49, 39:55, 41:04, 42:09, 44:17, 44:43:01, 44:45, 44:51, 44:107, 45:09, 46:01:03, 46:13:01, 46:21:02, 48:02:01-48:02:02, 49:04, 51:37, 51:45, 51:90, 51:92, 52:12, 53:01:01-53:01:02, 53:01:04- 53:06, 53:08:01, 53:09-53:18, 53:20- 53:24, 54:09, 54:14, 54:16, 55:14, 55:23, 55:27, 55:46, 56:09, 56:11, 56:18, 57:14, 58:01:01-58:01:04, 58:01:06-58:02, 58:04-58:07, 58:09- 58:19, 58:21-58:26, 58:29-58:31N, C*07:123, C*15:02:05, C*15:06:03
4	280 bp	1070 bp	*35:01:01:01- 35:01:22, 35:05:01- 35:05:02, 35:07- 35:08:04, 35:10- 35:11:02, 35:14:01- 35:17, 35:19-35:21, 35:23-35:30, 35:32, 35:35, 35:37, 35:40N-35:43:02, 35:45-35:54, 35:57- 35:58, 35:61-35:64, 35:66-35:69, 35:71- 35:72, 35:76-35:80, 35:82, 35:86, 35:89- 35:94, 35:97, 35:99- 35:105, 35:107- 35:108:02, 35:110- 35:126, 35:130N- 35:135, 35:137- 35:148	*07:09, 07:11, 07:17, 08:28, 08:35, 08:37, 13:04, 13:10, 13:26, 15:01:01:01-15:01:04, 15:01:06-15:08, 15:11:01-15:16, 15:18:01-15:21, 15:23- 15:29, 15:31-15:36, 15:38:01-15:40, 15:43-15:44, 15:46-15:47, 15:49-15:57, 15:60-15:62, 15:64-15:72, 15:74-15:76, 15:78:01-15:82, 15:84-15:85, 15:87- 15:89, 15:91-15:98, 15:101-15:129, 15:131-15:132, 15:134-15:136, 15:138- 15:149N, 15:151-15:161, 15:163- 15:167, 15:169-15:176, 15:178-15:187, 15:189-15:195, 15:197-15:207, 15:209N, 18:01:01-18:15, 18:17N- 18:25, 18:27-18:40, 18:42-18:52, 27:41, 39:07, 39:43, 40:03, 40:20, 40:38, 40:52, 40:59-40:60, 40:105, 40:158, 42:09, 44:17, 44:43:01-44:43:02, 45:09, 46:01:01-46:10, 46:12-46:17, 46:19- 46:26, 48:02:01-48:02:02, 48:14, 48:23, 49:04-49:05, 51:37, 51:45, 51:63, 51:97, 53:01:01-53:03, 53:05-53:06, 53:08:01-53:18, 53:20-53:24, 54:06,

Lot No.: **07L**

Lot-specific Information

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				54:09, 54:14, 55:14, 55:23, 55:32, 56:03, 56:09, 56:18, 57:01:01-57:01:10, 57:06, 57:08, 57:10-57:11, 57:13-57:16, 57:18-57:27, 57:29-57:31, 57:33-57:37, 58:01:01-58:02, 58:04-58:07, 58:09- 58:19, 58:21-58:26, 58:29-58:31N
5⁸	295 bp	800 bp	*35:02:01-35:02:04, 35:04:01-35:04:03, 35:06, 35:09:01- 35:09:02, 35:12:01- 35:12:02, 35:18, 35:22, 35:31, 35:44, 35:59-35:60, 35:81, 35:83, 35:87-35:88, 35:95, 35:129N, 35:146, 35:149	*07:05:01-07:06, 07:32, 07:34, 07:40, 07:53, 07:69, 07:78, 07:80, 07:90, 07:97, 07:105, 07:112, 08:01:01-08:05, 08:08N-08:13, 08:15-08:27, 08:29- 08:36, 08:38-08:48, 08:50-08:66, 13:01:01-13:01:02, 13:01:04-13:03, 13:06-13:09, 13:11-13:23, 13:25, 13:27- 13:33, 13:36-13:40, 14:13, 15:09, 15:30, 15:42, 15:48, 15:58, 15:73, 15:83, 15:86, 15:150, 15:188, 18:26, 27:07, 27:11, 27:20, 27:24, 27:27, 27:33, 27:43, 37:12, 38:01:01, 38:01:03-38:21, 38:23-38:26, 39:01:01:01-39:01:01:02L, 39:01:03- 39:06:02, 39:08-39:14, 39:16, 39:18- 39:20, 39:22-39:24, 39:26-39:28, 39:30- 39:42, 39:44-39:48, 39:50-39:54, 39:56- 39:62, 40:01:01-40:02:09, 40:02:11, 40:04-40:08, 40:10:01-40:16, 40:19, 40:21-40:23, 40:25-40:30, 40:34-40:40, 40:43, 40:45-40:51, 40:53-40:58, 40:61- 40:88, 40:90-40:97, 40:99-40:119, 40:121-40:123, 40:125-40:130, 40:132- 40:158, 41:01-41:03:02, 41:05-41:09, 41:11-41:16, 42:01:01-42:02, 42:04- 42:08, 42:10-42:14, 44:10, 44:14, 44:31, 44:62, 44:77, 44:82, 45:12, 46:11, 46:18, 48:01:01-48:01:03, 48:03:01-48:07, 48:09-48:16, 48:18- 48:22, 48:24, 51:01:01-51:01:18, 51:01:19 ^w , 51:01:20-51:02:02, 51:02:04- 51:16, 51:19-51:24:04, 51:26-51:36, 51:38-51:41N, 51:43-51:44N, 51:46, 51:48-51:84, 51:86-51:89, 51:91, 51:93, 51:95-51:106, 52:01:01-52:11, 52:13- 52:22, 53:19, 54:01-54:05N, 54:07- 54:08N, 54:10-54:13, 54:17-54:23, 55:01:01-55:05, 55:07-55:13, 55:15- 55:22, 55:24-55:26, 55:28-55:40, 55:42- 55:45, 55:47-55:48, 56:01:01-56:02, 56:04-56:08, 56:10, 56:12-56:17, 56:19N-56:30, 57:02:01-57:03:02,

Lot No.: **07L**

Lot-specific Information

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				57:07, 57:09, 57:12, 57:17, 57:28N, 58:08, 58:20, 58:27-58:28, 59:01-59:05, 67:01:01-67:03, 78:01-78:07, 81:01-81:05, 82:01-82:03
6⁶	85 bp	1070 bp	*35:02:01-35:02:04, 35:04:01-35:04:03, 35:12:01-35:12:02, 35:22, 35:34, 35:39, 35:44, 35:81, 35:83, 35:95, 35:129N, 35:149	*07:68:01-07:68:02, 07:100, 13:13, 13:35, 14:13, 15:09-15:10:02, 15:30, 15:37, 15:45, 15:48, 15:63, 15:83, 15:90, 15:99, 18:26, 27:06 ^w , 27:21 ^w , 27:27, 39:14, 39:29, 40:26, 40:28, 40:35, 40:87, 40:95, 40:121, 42:10, 44:05:01-44:05:03 ^w , 44:25 ^w , 44:42 ^w , 44:58N ^w , 44:62, 44:70 ^w , 44:75 ^w , 44:77, 44:78 ^w , 44:82, 44:107, 48:12, 51:01:01-51:01:14, 51:01:16-51:09:02, 51:11N-51:12, 51:16, 51:18-51:23, 51:26-51:36, 51:38-51:41N, 51:43-51:44N, 51:46, 51:48-51:61, 51:64-51:80, 51:82-51:84, 51:86-51:91, 51:93-51:96, 51:98N-51:105, 52:01:01-52:13, 52:15-52:22, 53:19, 54:04, 54:15 ^w , 55:17, 55:27-55:28, 56:05:01-56:06, 56:12, 56:15, 56:21, 57:02:01-57:03:02, 57:05, 57:07, 57:09, 57:12, 57:17, 57:28N, 58:08, 58:28, 78:01-78:07, C*01:32
7^{7,9,14}	160 bp, 330 bp	800 bp	*35:23, 35:84, 35:90, 35:150	*13:36, 15:06, 15:27:01-15:27:03, 15:84, 15:109, 15:195, 18:27, 27:23, 38:16, 40:08, 40:25, 40:68, 40:100, 40:106, 44:10, 44:12, 47:01:01:01-47:03, 47:06-47:07, 51:103, 73:01 ^w -73:02 ^w , 82:01-82:02
8	280 bp	1070 bp	*35:03:01-35:03:07, 35:06, 35:13, 35:33, 35:36, 35:38, 35:55-35:56, 35:59-35:60, 35:65Q, 35:70, 35:74-35:75, 35:84-35:85, 35:96, 35:98, 35:106, 35:109, 35:127-35:128, 35:136, 35:150-35:151	*07:78, 13:01:01-13:03, 13:06-13:09, 13:11-13:12, 13:14-13:20, 13:22:01-13:23, 13:25, 13:27-13:34, 13:36-13:40, 15:42, 15:58, 15:73, 15:86, 15:133, 38:01:01-38:26, 39:01:01:01-39:01:01:02L, 39:01:03-39:06:02, 39:08-39:13:02, 39:15-39:16, 39:18-39:20, 39:22-39:24, 39:26-39:28, 39:30-39:42, 39:44-39:62, 40:36, 40:48, 40:71, 40:77, 40:86, 42:06, 44:10, 46:11, 46:18, 48:07, 51:13:01-51:13:02, 51:15, 51:62, 51:92, 51:106, 52:14, 53:04, 54:01-54:03, 54:05N, 54:07-54:08N, 54:10, 54:12-54:13, 54:16-54:23, 55:01:01-55:03, 55:05, 55:07, 55:09-55:13, 55:15-55:16, 55:18-55:19, 55:21-55:22, 55:24-55:26, 55:29-55:31, 55:33-55:40, 55:42-55:48,

				56:01:01-56:02, 56:04, 56:07-56:08, 56:10-56:11, 56:13-56:14, 56:16-56:17, 56:19N-56:20, 56:22-56:30, 58:20, 59:01, 59:03-59:05, 67:01:01-67:03, 82:01-82:03
9	140 bp	800 bp	*35:05:01-35:05:02, 35:16-35:17, 35:22, 35:30, 35:51, 35:58, 35:66, 35:72, 35:89, 35:97, 35:113, 35:125	*07:68:01-07:68:02, 07:100, 14:05, 14:13, 15:07:01-15:07:02, 15:45, 15:55, 15:68, 15:126, 15:207, 18:14, 18:22, 27:32, 38:19, 39:03, 39:14, 39:24, 39:29, 39:37, 40:35, 40:71, 42:10, 44:54, 44:106, 44:110, 46:12, 48:12, 48:14, 51:64, 53:14, 58:18
10^{9,15}	140 bp, 240 bp, 310 bp	1070 bp	*35:07, 35:83, 35:94	C*07:63
11^{9,10}	225 bp	800 bp	*35:08:01-35:08:04, 35:18, 35:45, 35:61, 35:80, 35:100, 35:105, 35:142	*07:12, 07:14, 07:18:01-07:18:02, 44:04, 44:28:01-44:28:02, 44:56N, 44:76, 53:11, 53:16, 53:24, 57:02:01-57:02:02, 57:04-57:05, 57:12-57:13, 57:19, 57:28N, 57:30, 58:22, A*01:41^w, C*03:28, C*03:49, C*04:80, C*05:10, C*15:08
12^{6,16}	95 bp, 125 bp	1070 bp	*35:10, 35:13, 35:16, 35:28, 35:69, 35:80, 35:92	*15:202, 18:12, 37:01:01-37:01:05, 37:01:07-37:05, 37:07, 37:09-37:25, 44:83, 49:03, 52:01:01-52:01:06, 52:03-52:15, 52:17, 52:19-52:22, 53:17, 78:05-78:06
13	170 bp	800 bp	*35:11:01-35:11:02, 35:14:01-35:14:02, 35:21, 35:43:01-35:44, 35:58, 35:67, 35:79, 35:86, 35:96, 35:99, 35:102, 35:117-35:118, 35:135	*07:02:01-07:24, 07:26-07:47, 07:49N-07:50, 07:52-07:117, 08:20, 08:53, 13:16, 13:20, 13:31, 14:01:01-14:19, 15:01:01:01-15:01:04, 15:01:06-15:04, 15:06-15:19, 15:21, 15:23-15:30, 15:32-15:40, 15:42-15:47, 15:49-15:50, 15:53-15:54, 15:56-15:58, 15:60-15:74, 15:76-15:82, 15:85, 15:87, 15:89-15:90, 15:92-15:99, 15:101-15:104, 15:106, 15:108-15:110, 15:112-15:113, 15:115-15:122, 15:125-15:129, 15:131-15:135, 15:137-15:144, 15:146-15:150, 15:152-15:154, 15:156-15:161, 15:163-15:175, 15:177-15:178, 15:180-15:184, 15:187, 15:189-15:199, 15:201-15:209N, 18:15, 18:19, 18:21, 18:30, 27:04:01-27:04:03, 27:06, 27:10, 27:15, 27:18, 27:20-27:21, 27:24-27:25, 27:40, 27:54, 27:63, 27:66N, 27:68-27:69, 37:07,

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

				37:13, 38:10, 39:18, 39:35-39:36, 40:05, 40:15-40:16, 40:23, 40:26, 40:28, 40:32, 40:51, 40:95, 40:98, 40:148, 40:158, 44:76, 44:79, 46:01:01-46:05, 46:07N-46:08, 46:10, 46:12, 46:14-46:17, 46:20, 46:22-46:24, 48:05, 48:08, 48:15, 49:01:01-49:10, 49:12-49:15, 50:01:01-50:02, 50:04-50:12, 51:01:01-51:04, 51:06-51:07:02, 51:11N-51:14, 51:16-51:18, 51:21-51:24:04, 51:26-51:30, 51:32-51:39, 51:41N, 51:43, 51:45-51:46, 51:48-51:52, 51:55-51:72, 51:74-51:80, 51:82-51:92, 51:94-51:96, 51:98N-51:106, 52:01:01-52:02, 52:04-52:09, 52:11-52:18, 52:20-52:22, 53:06, 53:08:01-53:08:02, 54:06, 54:20, 55:01:01-55:01:06, 55:03, 55:05, 55:09, 55:11, 55:14-55:15, 55:17, 55:21, 55:24-55:25, 55:28-55:29, 55:31, 55:33, 55:36, 55:38, 55:40, 55:44-55:45, 56:03, 56:05:01-56:06, 56:21, 56:25, 57:09, 57:13, 57:22, 58:06, 58:08, 58:19, 78:01-78:03, 78:05-78:07
14 ^{6,8,9,10}	75 bp	800 bp	*35:01:17, 35:03:07, 35:04:03, 35:12:01-35:12:02, 35:39, 35:59, 35:61, 35:88, 35:149	*07:14, 07:18:01-07:18:02, 08:60, 13:01:01-13:01:05, 13:06-13:07N, 13:12-13:13, 13:17, 13:20-13:23, 13:25, 13:28-13:29, 13:36, 13:39, 14:10, 15:02:01-15:02:05, 15:13, 15:21, 15:25:01-15:25:03, 15:36, 15:44, 15:77, 15:80, 15:88-15:89, 15:106, 15:112, 15:121, 15:139, 15:144, 15:154, 15:170, 15:204, 27:19, 27:30, 38:20, 39:42, 40:04, 40:28, 40:30, 40:34, 40:64, 40:68, 40:129, 40:137, 42:13, 44:02:01:01-44:02:05, 44:02:07-44:02:11, 44:02:13-44:09, 44:11-44:14, 44:16-44:17, 44:19N, 44:21-44:30, 44:32-44:35, 44:37-44:40, 44:42-44:46, 44:48-44:53, 44:55-44:64:02, 44:66-44:81, 44:83-44:86, 44:88-44:98, 44:101-44:105, 44:107-44:115, 51:04, 51:42, 51:46, 51:56, 53:17, 55:14, 56:12, 57:01:01-57:04, 57:06-57:10, 57:12-57:20, 57:22-57:32, 57:34-57:37, 58:28, 83:01
15 ⁶	135 bp	1070 bp	*35:15, 35:33, 35:66, 35:86	*07:68:01-07:68:02, 13:01:01-13:02:09, 13:07N-13:11, 13:13-13:20,

				13:22:01-13:23, 13:25-13:40, 15:40, 15:47, 15:49, 15:52, 15:114, 15:117, 15:124, 15:138, 18:10, 18:35, 18:50, 27:01-27:06, 27:08-27:10, 27:12-27:14, 27:16-27:19, 27:21, 27:23, 27:26-27:27, 27:29-27:32, 27:35, 27:37, 27:39-27:42, 27:44-27:46, 27:48-27:61, 27:64N-27:70, 27:72-27:73, 37:02, 39:47, 39:50, 40:35, 40:77, 40:87, 40:95, 40:121, 40:158, 44:16, 44:21, 44:64:01-44:64:02, 45:10, 47:01:01:01-47:07, 48:12, 48:14, 49:07, 51:16, 51:31, 51:34, 51:82, 51:93, 52:08, 55:09, 55:22, 55:24, 57:25
16^{6,17}	95 bp, 190 bp	1070 bp	*35:19, 35:47, 35:77	*15:143, 18:48, 40:08, 40:25, 40:68, 40:106, 44:12
17^{9,11, 18}	180 bp, 385 bp	1070 bp	*35:26, 35:36	*14:11, 15:189, 39:04
18^{6,9,19}	105 bp, 180 bp	800 bp	*35:20:01-35:20:02, 35:28, 35:35, 35:72, 35:93, 35:95	*08:37, 15:50, 15:69, 15:93, 15:186, 15:199, 15:202, 18:01:01-18:15, 18:17N-18:52, 37:01:01-37:01:05, 37:01:07-37:05, 37:07-37:20, 37:22-37:25, 39:07, 39:43, 42:09, 44:83, 46:03, 46:21:01-46:21:02, 49:03, 51:07:01-51:07:02, 52:01:01-52:01:06, 52:03-52:05, 52:07-52:15, 52:17-52:21, 53:09, 53:17, 54:09, 54:14, 55:14, 55:23, 56:18, 57:26, 58:05, 78:05-78:06, C*07:123, C*15:09, C*15:19-15:20, C*15:27, C*16:20
19^{9,10}	230 bp	1070 bp	*35:29, 35:69, 35:74, 35:76, 35:136	*07:29, 07:65, 07:72, 07:86, 08:01:01-08:01:13, 08:02 ^w , 08:04-08:05, 08:07-08:22, 08:24-08:53, 08:55-08:65, 14:11, 15:61, 15:70, 15:76, 15:101, 15:180, 15:189, 39:04, 40:43, 40:73, 44:90, 45:06, 46:01:01-46:04, 46:06-46:26, 51:20 ^w , 53:23 ^w , 54:01, 54:03-54:11, 54:12 ^w , 54:13-54:22, 55:01:01-55:05, 55:07-55:09, 55:11-55:17, 55:19-55:30, 55:32-55:48, 56:01:01-56:06, 56:07 ^w , 56:08-56:20, 56:21 ^w , 56:22, 56:24-56:30, 78:01, 78:03, 78:07, 83:01
20¹⁰	200 bp	1070 bp	*35:31, 35:88	*07:05:01-07:06, 07:32, 07:40, 07:53, 07:69, 07:78, 07:80, 07:90, 07:97, 07:105, 07:112, 08:40, 13:01:01-13:01:02, 13:01:04-13:02:09,

				13:07N-13:09, 13:11, 13:13-13:23, 13:25, 13:27-13:33, 13:36-13:40, 27:07, 27:11, 27:20, 27:24, 27:27, 27:33, 27:43, 39:47, 39:50, 40:01:01-40:02:09, 40:02:11, 40:04, 40:06:01:01-40:08, 40:10:01-40:16, 40:19, 40:21-40:23, 40:25, 40:27, 40:29-40:30, 40:34-40:38, 40:40, 40:43, 40:45-40:50, 40:53-40:58, 40:61-40:62, 40:64-40:70, 40:72:01-40:88, 40:90-40:91, 40:93-40:97, 40:99-40:119, 40:121-40:123, 40:125-40:130, 40:132, 40:134-40:158, 44:06, 44:31, 48:01:01-48:01:03, 48:03:01-48:07, 48:09-48:16, 48:18-48:22, 48:24, 49:03, 51:01:01, 51:01:03-51:01:08, 51:01:10-51:02:01, 51:02:03-51:04, 51:06-51:07:01, 51:08-51:09:01, 51:10-51:14, 51:16-51:24:04, 51:26-51:42, 51:44N-51:46, 51:48-51:53, 51:55-51:77, 51:79-51:88, 51:90-51:102, 51:104-51:106, 52:01:02, 52:01:04, 52:02-52:03, 52:06:01-52:06:02, 52:08-52:09, 52:21, 53:06, 55:09, 55:22, 55:24, 58:08, 58:27, 81:01-81:05
21⁹	260 bp	800 bp	*35:25, 35:124-35:125, 35:142	*18:01:01-18:03, 18:05-18:15, 18:17N-18:28, 18:30-18:52, 37:01:01-37:21, 37:23-37:25, 40:149 [?] , 52:16, 58:12, 81:01, 81:03 [?] -81:05 [?] , 83:01
22^{6,20}	90 bp, 270 bp	1070 bp	*35:21, 35:24:01-35:24:02, 35:46, 35:81, 35:96, 35:109	*13:36, 14:10, 15:02:01-15:02:05, 15:08, 15:11:01-15:11:05, 15:13, 15:15, 15:21, 15:31, 15:44, 15:55, 15:76 ^w , 15:88-15:89, 15:112, 15:121, 15:139, 15:144, 15:148, 15:170, 15:189, 15:191, 15:194, 15:209N, 18:22, 37:04:01-37:04:02, 40:28, 51:04, 51:46, 51:56, 51:104, 53:02, 53:06, 55:35 ^w , 57:14, 58:09, C*15:39
23	190 bp	800 bp	*35:01:01:01-35:10, 35:13, 35:15, 35:18-35:20:02, 35:23-35:30, 35:33-35:37, 35:40N-35:42:02, 35:45-35:50, 35:52-35:57, 35:60, 35:63-35:66, 35:68:01-35:72, 35:74-35:78, 35:80-35:85, 35:88-35:95, 35:97-35:98,	*13:01:01-13:04, 13:06-13:09, 13:11-13:15, 13:17-13:19, 13:22:01-13:23, 13:25-13:30, 13:32-13:34, 13:36-13:40, 15:20, 15:86, 18:22, 18:52, 27:14, 40:04, 40:24, 40:59-40:60, 40:68, 40:70, 40:99, 40:120, 40:125, 40:129, 44:10, 46:11, 46:18, 48:02:01-48:02:02, 48:17, 49:11, 51:15, 53:01:01-53:05, 53:09-53:21, 53:23, 53:24 ^w , 54:01-54:05N, 54:07-54:08N, 54:10-54:19, 54:21-54:23, 55:02:01-55:02:06, 55:07,

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

			35:100-35:101:02, 35:103-35:112, 35:114, 35:116, 35:119-35:124, 35:126-35:134N, 35:136-35:140, 35:142-35:148, 35:150-35:151	55:10, 55:12-55:13, 55:16, 55:18-55:19, 55:22-55:23, 55:26-55:27, 55:30, 55:32, 55:34-55:35, 55:37, 55:39, 55:41-55:43, 55:46-55:48, 56:01:01-56:02, 56:07- 56:11, 56:14-56:17, 56:19N-56:20, 56:22-56:24, 56:26, 56:28N-56:29, 57:05, 58:01:01-58:02, 58:04, 58:07, 58:09, 58:11-58:18, 58:21-58:26, 58:28- 58:31N, 59:01-59:05, C*02:03, C*04:34, C*05:12, C*07:95, C*08:39
24¹⁰	425 bp	1070 bp		*27:02-27:03, 27:05:02-27:05:15, 27:07, 27:09-27:10, 27:13-27:14, 27:16-27:17, 27:19, 27:27-27:30, 27:32, 27:34-27:35, 27:37-27:39, 27:41, 27:43, 27:45-27:48, 27:50-27:60, 27:62, 27:64N-27:65N, 27:67, 27:70-27:73, 37:01:01-37:04:02, 37:06-37:09, 37:12-37:13, 37:15-37:25, 40:13, 40:19 [?] , 40:109 [?] , 40:117 [?] , 44:06, 44:25, 44:50, 44:95, 47:01:01:01- 47:01:01:02, 47:05-47:07, 51:01:01- 51:24:04, 51:26-51:46, 51:48-51:53, 51:55-51:77, 51:79-51:106, 52:01:01- 52:19, 52:21-52:22, 53:01:01-53:08:02, 53:10, 53:14-53:24, 57:01:01-57:11, 57:13-57:37, 58:01:01-58:02, 58:04- 58:16, 58:18-58:31N
25^{6,8,21}	135 bp, 220 bp	800 bp	*35:09:02, 35:18, 35:27, 35:31, 35:56, 35:64, 35:88, 35:114, 35:118	*07:02:01-07:02:13, 07:02:15-07:67N, 07:69-07:99, 07:101-07:117, 08:01:02, 08:01:13, 08:20, 13:21, 15:02:03, 15:03:02, 15:33, 15:78:02, 15:107, 15:116, 15:141, 15:150, 15:188, 18:21, 18:49, 27:01-27:21, 27:23-27:73, 37:02, 37:05, 37:07, 37:09, 38:22, 40:01:01- 40:01:13, 40:01:15-40:13, 40:14:03- 40:16, 40:18-40:25, 40:27, 40:29-40:40, 40:42-40:70, 40:72:02-40:76, 40:78- 40:81, 40:83-40:86, 40:88-40:94, 40:96- 40:120, 40:122-40:135, 40:137-40:157, 41:08, 44:31, 46:06, 47:01:01:01-47:07, 48:01:01-48:01:03, 48:03:01-48:11, 48:13, 48:15-48:24, 51:01:09, 51:10, 51:24:02-51:24:04, 51:81, 54:11, 55:04, 55:08, 57:01:07, 57:13, 58:27, 67:03, 78:06, 81:01-81:05
26²²	210 bp, 370 bp	800 bp	*35:42:01, 35:50, 35:84	*07:08, 07:32, 07:38, 18:07:01- 18:07:02, 18:29, 27:23, 37:21,

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

				38:06-38:07, 39:58, 40:08, 40:13, 40:68, 40:106 [?] , 48:06, 51:57, 51:103, 53:21
27 ^{6,9,23}	110 bp, 160 bp	1070 bp	*35:41, 35:84, 35:90, 35:150	*13:36, 27:23, 38:16, 40:08, 40:25, 40:68, 40:106, 44:12, 51:103, 73:01 ^w - 73:02 ^w
28	230 bp	800 bp	*35:05:01-35:05:02, 35:22, 35:32, 35:43:01-35:44, 35:51, 35:58, 35:67, 35:68:02, 35:72, 35:79, 35:89, 35:97, 35:99, 35:102, 35:114, 35:117- 35:118, 35:135, 35:151	*07:20, 07:24, 07:60, 07:100, 08:21, 08:25, 15:01:01:01-15:01:04, 15:01:06- 15:01:13, 15:01:15-15:01:20, 15:03:01- 15:03:03, 15:05:01-15:05:02, 15:07:01- 15:11:02, 15:11:04-15:11:05, 15:12 ^w , 15:14-15:15, 15:17:01:01-15:18:01, 15:18:03-15:18:04, 15:19 ^w , 15:23-15:24, 15:28-15:31, 15:33-15:35, 15:37- 15:39:02, 15:43, 15:45-15:46, 15:48, 15:51, 15:53-15:58, 15:60-15:61, 15:63- 15:66, 15:68, 15:70-15:76, 15:78:01- 15:79N, 15:81-15:82, 15:87, 15:90- 15:92, 15:94N, 15:96-15:99, 15:101- 15:105, 15:107-15:108, 15:111N, 15:113, 15:115-15:116, 15:118-15:120, 15:122-15:123, 15:125-15:128, 15:131- 15:136, 15:140-15:143, 15:145-15:153, 15:156-15:169, 15:171-15:183, 15:185, 15:187, 15:189-15:193, 15:196-15:198, 15:200-15:203, 15:205-15:209N, 18:36, 39:32, 40:05, 40:26, 40:63, 40:71, 40:92, 44:41, 44:54, 44:65, 44:82, 44:106, 46:01:01-46:02, 46:04-46:05, 46:08-46:10, 46:12-46:17, 46:19-46:20, 46:22-46:26, 51:06, 51:45, 51:59, 51:62, 51:64, 53:14, 54:06, 55:08, 56:02-56:04, 58:16, 58:18, C*03:05, C*03:25, C*03:27
29 ⁶	105 bp	1070 bp	*35:37	*13:04, 15:04, 15:16, 15:67, 15:95, 15:137, 15:155, 39:06:01-39:06:02, 39:33-39:34, 39:50, 39:57, 39:62, 40:86, 45:02, 45:09, 49:04-49:05, 50:09, 51:13:01-51:13:02, 51:37, 51:63, 51:92, 51:97, 52:14, 54:14, 55:13, 55:23, 55:32, 56:22, 59:03, 73:01-73:02
30 ^{6,9,10,24}	115 bp, 325 bp	800 bp	*35:15, 35:25-35:26, 35:33, 35:49, 35:66	*07:02:13, 07:68:02, 08:40, 13:01:01- 13:02:09, 13:07N-13:11, 13:13-13:21, 13:22:02-13:23, 13:25-13:40, 18:10, 18:35, 18:37, 18:50, 27:01-27:21, 27:23-27:51, 27:53-27:66N, 27:68- 27:73, 39:47, 39:50, 40:14:01, 40:46 [?] ,

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

				40:72:01, 40:77, 40:93 [?] , 40:95, 40:121, 40:136, 44:16, 44:21, 44:40, 44:44, 44:64:01-44:64:02, 45:10, 48:12, 49:07, 51:16, 51:31, 51:34, 51:82, 51:93, 52:08, 52:16, 55:09, 55:22, 55:24, 81:01, 81:03 [?] -81:05 [?] , 83:01, C*01:10^w , C*02:02:14^w , C*07:15^w
31 ^{9,25}	170 bp, 210 bp	1070 bp	*35:25, 35:50, 35:84, 35:124- 35:125, 35:142	*07:08, 07:32, 07:38, 08:01:01-08:03, 08:05, 08:07-08:16, 08:18-08:25, 08:27-08:53, 08:55, 08:57-08:60, 08:62-08:64, 08:66, 15:29, 15:180, 18:07:01-18:07:02, 18:29, 27:23, 37:21, 38:06-38:07, 39:58, 40:08, 40:13, 40:68, 48:06, 51:57, 51:103, 53:21
32 ¹⁰	170 bp	1070 bp	*35:29, 35:69, 35:76, 35:136	*07:02:01-07:02:02, 07:02:04-07:05:03, 07:05:05-07:15, 07:17-07:26, 07:28-07:36, 07:39-07:49N, 07:51-07:55, 07:57-07:72, 07:74, 07:76-07:90, 07:92-07:104, 07:106-07:108, 07:110-07:117, 08:01:11, 08:56, 15:70, 15:76, 15:101, 15:189, 18:03 ^w , 39:19:02, 42:01:01-42:02, 42:04-42:10, 42:12-42:14, 46:01:01, 46:01:03-46:01:05, 46:03-46:26, 51:20, 53:23, 55:35, 56:03, 56:06, 73:01-73:02, 78:01, 78:03, 78:07, 81:01-81:05, 83:01
33 ^{6,26}	70 bp, 205 bp, 310 bp	1070 bp	*35:45, 35:47, 35:71, 35:94	*07:20, 15:14, 15:46, 15:91, 15:106, 15:131, 15:161, 40:02:04, 40:10:02, 40:132, 44:10, 44:14, 44:17, 44:31, 44:43:01-44:43:02, 44:45, 44:51, 44:62, 44:77, 44:82, 44:84, 44:107, 45:09, 46:17, 51:23, 52:15, 53:22, 57:07, 58:07, 82:01-82:03
34	230 bp	800 bp	*35:48	*53:07, C*03:90
35 ^{8,9,27}	240 bp, 365 bp	1070 bp	*35:40N, 35:68:01	*08:12:03
36 ^{6,28}	110 bp, 180 bp, 230 bp	800 bp	*35:35, 35:70, 35:79	*38:20, 39:42, 58:30
37 ⁶	85 bp	1070 bp	*35:60	*13:01:01-13:03, 13:06-13:09, 13:11-13:12, 13:14-13:20, 13:22:01-13:23, 13:25, 13:27-13:34, 13:36-13:40, 15:42, 15:73, 15:86, 39:17, 40:71, 44:10, 44:15, 44:18, 45:01, 45:04-45:08, 45:10-45:13, 46:11, 46:18,

				49:01:01-49:03, 49:06-49:15, 50:01:01-50:02, 50:04-50:08, 50:10-50:12, 51:15, 51:62, 51:106, 54:01-54:03, 54:05N, 54:07-54:08N, 54:10, 54:12-54:13, 54:17-54:23, 55:01:01-55:03, 55:05, 55:07, 55:09-55:12, 55:15-55:16, 55:18-55:19, 55:21-55:22, 55:24-55:26, 55:29-55:31, 55:33-55:45, 55:47-55:48, 56:01:01-56:01:03, 56:02, 56:04, 56:07-56:08, 56:10, 56:13-56:14, 56:16-56:17, 56:19N, 56:23-56:30, 59:01, 59:04-59:05, 82:01-82:03
38⁸	300 bp	800 bp	*35:02:01-35:02:04, 35:81, 35:83, 35:95, 35:129N, 35:146	*45:12, 53:19
39⁶	115 bp	1070 bp	*35:14:01-35:14:02, 35:43:01-35:44, 35:62, 35:67, 35:79, 35:86, 35:102, 35:117, 35:135	*07:100, 13:31, 15:01:01:01-15:01:04, 15:01:06-15:01:16, 15:01:18-15:01:20, 15:04, 15:07:01-15:08, 15:11:01-15:12, 15:14-15:15, 15:19, 15:24, 15:26N-15:28, 15:30, 15:32, 15:34-15:35, 15:38:01-15:38:02, 15:43, 15:45-15:46, 15:50, 15:53-15:54, 15:56-15:58, 15:60, 15:63, 15:66, 15:68, 15:70-15:71, 15:73, 15:75-15:77, 15:79N, 15:81-15:82, 15:85, 15:87, 15:92, 15:94N, 15:96-15:97, 15:101-15:102, 15:104-15:105, 15:109-15:111N, 15:113, 15:117-15:118, 15:120, 15:122, 15:125-15:126, 15:128-15:129, 15:135, 15:137, 15:140, 15:142-15:149N, 15:152, 15:154, 15:157, 15:159-15:160, 15:163-15:167, 15:169, 15:171-15:172, 15:174-15:175, 15:178, 15:180-15:184, 15:187, 15:189-15:193, 15:201-15:203, 15:205-15:207, 15:209N, 18:19, 27:25, 39:36, 46:01:01-46:02, 46:04-46:05, 46:07N, 46:09-46:10, 46:12, 46:14-46:17, 46:20, 46:22-46:24, 51:61, 52:21, 54:06, 55:21, 56:03, A*68:56, C*06:20, C*12:50
40	185 bp	1070 bp	*35:63, 35:137, 35:147	*07:73, 15:53, 27:08, 27:12, 27:18, 27:26, 27:33, 27:40, 27:42, 27:44, 40:01:01-40:02:03, 40:02:05-40:09, 40:11:01-40:11:02, 40:14:01-40:16, 40:18, 40:20, 40:22N-40:40, 40:42-40:46, 40:48-40:75, 40:77-40:82, 40:84-40:93, 40:95, 40:97-40:108, 40:111-40:116, 40:118N-40:136,

				40:138-40:156, 40:158, 41:01-41:04, 41:06-41:11, 41:13-41:16, 44:09, 44:46, 44:75, 44:90, 45:01-45:13, 47:02, 47:03 ^W , 50:01:01-50:02, 50:04-50:12
41 ^{6,29}	105 bp, 130 bp	1070 bp	*35:52, 35:82, 35:85, 35:92, 35:135	*18:33, 39:19:01-39:19:02, 51:22, 78:03
42 ^{6,30}	115 bp, 155 bp, 190 bp, 250 bp	800 bp	*35:53N, 35:65Q, 35:91, 35:97	
43 ³¹	205 bp, 255 bp	1070 bp	*35:54, 35:89	
44 ^{6,32}	110 bp, 145 bp, 300 bp	1070 bp	*35:55, 35:78	*18:09, 37:10, 44:83, 51:54, 51:78, 52:20, 53:09, 53:11-53:13
45 ¹³	165 bp	800 bp	*35:01:01:01-35:30, 35:32-35:42:02, 35:44-35:56, 35:58- 35:59, 35:61-35:72, 35:74-35:78, 35:80- 35:86, 35:88-35:151	*15:20, 48:02:01-48:02:02, 51:01:01- 51:24:04, 51:26-51:41N, 51:43-51:46, 51:48-51:106, 52:01:01-52:06:02, 52:08-52:22, 53:01:01-53:24, 56:05:01- 56:05:02, 56:21, 58:01:01-58:02, 58:04- 58:10N, 58:12-58:19, 58:21-58:31N, 78:01-78:07, 81:03, 83:01 [?]
46 ^{8,9,12,13}	165 bp	1070 bp	*35:31, 35:35, 35:43:01-35:43:02, 35:46, 35:57, 35:60, 35:79, 35:87	*07:02:01-07:57, 07:59-07:117, 08:01:01-08:05, 08:07-08:66, 13:01:01- 13:04, 13:06-13:23, 13:25-13:40, 14:01:01-14:19, 15:01:01:01-15:01:04, 15:01:06-15:19, 15:21, 15:23-15:40, 15:42-15:58, 15:60-15:99, 15:101, 15:103-15:129, 15:131-15:209N, 18:01:01-18:15, 18:17N-18:52, 27:01- 27:21, 27:23-27:73, 37:01:01-37:25, 38:01:01-38:26, 39:01:01:01- 39:01:01:02L, 39:01:03-39:20, 39:22- 39:45, 39:47-39:62, 40:01:01-40:16, 40:18-40:40, 40:42-40:158, 41:01- 41:16, 42:01:01-42:02, 42:04-42:14, 44:02:01:01-44:65, 44:67-44:115, 45:01-45:13, 46:01:01-46:26, 47:01:01:01-47:07, 48:01:01-48:01:03, 48:02:02-48:24, 49:01:01-49:15, 50:01:01-50:02, 50:04-50:12, 51:42, 52:07, 54:01-54:23, 55:01:01-55:05, 55:07-55:48, 56:01:01-56:04, 56:06- 56:20, 56:22-56:30, 57:01:01-57:37, 58:11, 58:20, 59:01-59:05,

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

				67:01:01-67:03, 81:01-81:02, 81:04N-81:05, 82:01-82:03, 83:01 [?]
47	160 bp	800 bp	*35:38	*42:06, 56:13, 82:01-82:03, C*07:04:01^w-07:04:04^w, C*07:11^w-07:12^w, C*07:45^w, C*07:63^w, C*07:101^w, C*07:139^w, C*07:142^w
48	390 bp	1070 bp	*35:01:01:01-35:19, 35:21-35:25, 35:27, 35:29-35:62, 35:64-35:71, 35:74-35:75, 35:77, 35:79-35:81, 35:83-35:84, 35:86-35:94, 35:96-35:134N, 35:136-35:151	*18:07:01-18:07:02, 18:29, 27:23, 37:21, 40:08, 40:13, 40:68 [?] , 40:106 [?] , 44:06, 51:01:01-51:06, 51:08-51:21, 51:23-51:24:04, 51:26-51:46, 51:48-51:106, 52:06:01-52:06:02, 53:01:01-53:08:02, 53:10-53:16, 53:18-53:24, 78:01-78:02:02, 78:04, 78:07
49	385 bp	1070 bp	*35:01:04, 35:05:01-35:05:02, 35:08:03, 35:30, 35:58, 35:66, 35:68:02, 35:89, 35:97, 35:114, 35:151	*51:56, 53:14
50	180 bp	1070 bp	*35:101:01-35:101:02	*51:76
51^{6,33}	65 bp, 160 bp	1070 bp	*35:100, 35:102, 35:145N	
52³⁴	220 bp, 250 bp	1070 bp	*35:104, 35:141	
53^{6,9,35}	135 bp, 220 bp	1070 bp	*35:103, 35:129N	
54³⁶	160 bp, 190 bp	1070 bp	*35:106, 35:110	
55⁶	115 bp	1070 bp	*35:108:01-35:108:02	*08:59
56³⁷	140 bp, 215 bp,	1070 bp	*35:107, 35:128	
57³⁸	150 bp, 185 bp	800 bp	*35:111, 35:149	
58^{6,39}	110 bp, 160 bp, 200 bp	1070 bp	*35:112, 35:115, 35:139	*08:37, 15:51, 15:179, 15:199, 42:09, 44:17, 45:09, 53:22, 57:24, C*05:30
59^{6,40}	75 bp, 150 bp	1070 bp	*35:116, 35:127	*57:21
60^{6,8,41}	90 bp, 135 bp	800 bp	*35:120-35:121	

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

61 ^{6,42}	110 bp, 175 bp, 265 bp	1070 bp	*35:122-35:123, 35:139	*56:29, C*05:30
62 ⁴³	140 bp, 260 bp	1070 bp	*35:132, 35:144	
63 ⁶	65 bp	1070 bp	*35:146	C*06:21
64 ^{6,44}	90 bp, 245 bp	1070 bp	*35:130N, 35:134N	
65 ^{6,9,45}	100 bp, 220 bp	1070 bp	*35:131, 35:148	
66 ^{6,46}	90 bp, 115 bp	1070 bp	*35:105, 35:113, 35:133	
67 ⁶	105 bp	1070 bp	*35:138	*44:26, 47:07
68 ⁶	90 bp	1070 bp	*35:137, 35:140, 35:147	*27:52, 51:103
69 ^{6,47}	95 bp, 325 bp	1070 bp	*35:143, 35:147	*51:73, 55:26
70	140 bp	1070 bp	*35:117	*15:197
71 ^{6,48}	120 bp, 160 bp	800 bp	*35:119, 35:126	
72	220 bp	1070 bp	*35:09:02, 35:18, 35:31, 35:64, 35:88, 35:114, 35:118	*07:02:01-07:02:13, 07:02:15-07:67N, 07:69-07:99, 07:101-07:117, 08:01:02, 08:01:13, 08:20, 13:21, 15:02:03, 15:03:02, 15:33, 15:78:02, 15:107, 15:116, 15:141, 15:150, 15:188, 18:21, 27:01-27:21, 27:23-27:73, 37:02, 37:07, 37:09, 38:22, 40:01:01-40:01:13, 40:01:15-40:13, 40:14:03-40:16, 40:18- 40:25, 40:27, 40:29-40:40, 40:42-40:70, 40:72:02-40:76, 40:78-40:81, 40:83- 40:86, 40:88-40:94, 40:96-40:120, 40:122-40:135, 40:137-40:157, 41:08, 44:31, 46:06, 47:01:01:01-47:07, 48:01:01-48:01:03, 48:03:01-48:11, 48:13, 48:15-48:24, 51:01:09, 51:10, 51:24:02-51:24:04, 51:81, 54:11, 55:04, 55:08, 57:01:07, 57:13, 58:27, 67:03, 81:01-81:05

¹Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of HLA-B*35 SSP typings. When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective lengths of the specific PCR product(s) of the alleles amplified by these primer mixes are given. Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*35 subtyping.

In addition, wells number 3, 5, 7, 9, 11, 13, 14, 18, 21, 23, 25, 26, 28, 30, 34, 36, 42, 45, 47, 57, 60 and 71 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

In the presence of a specific amplification the intensity of the control band often decreases.

³For several HLA-B alleles first exon nucleotide sequences are not available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. We assume that unknown sequences in the first exon are conserved within allelic groups.

⁴The B*35:122 and B*35:123 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 61.

The B*35:132 and B*35:144 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 62.

The B*35:130N and B*35:134N alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 64.

The B*35:131 and B*35:148 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 65.

⁵Due to the sharing of sequence motifs many non-HLA-B*35 alleles will be amplified by the HLA-B*35 primer mixes. In addition, the A*01:41 allele will be weakly amplified by primer mix 11. Furthermore, the A*68:56 and the C*06:20 and C*12:50 alleles will be amplified by primer mix 39, the C*01:10, C*02:02:14 and C*07:15 alleles will be weakly amplified by primer mix 30, the C*01:32 allele is weakly amplified by primer mix 6, the C*02:03, C*04:34, C*05:12, C*07:95 and C*08:39 alleles will be amplified by primer mix 23, the C*03:05, C*03:25 and C*03:27 alleles will be amplified by primer mix 28, the C*03:28, C*03:49, C*04:80, C*05:10 and C*15:08 alleles will be amplified by primer mix 11, the C*03:90 allele will be amplified by primer mix 34, the C*03:102 allele will be amplified by primer mix 2, the C*05:30 allele will be amplified by primer mixes 58 and 61, the C*06:21 allele will be amplified by primer mix 63, the C*07:04:01-07:04:04, C*07:11-07:12, C*07:45, C*07:101, C*07:139 and 07:142 alleles will be weakly amplified by primer mix 47, the C*07:63 allele will be amplified by primer mixes 10 and weakly by primer mix 47, the C*07:123 allele will be amplified by primer mixes 3 and 18, the C*15:02:05 and C*15:06:03 alleles will be amplified by primer mix 3, the C*15:09, C*15:19-15:20, C*15:27 and C*16:20 alleles will be amplified by primer mix 18 and the C*15:39 allele will be amplified by primer mix 22.

⁶Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁷Primer mix 7 may faintly amplify the B*44 alleles.

⁸Primer mixes 5, 9, 25, 35, 38, 46 and 60 may give rise a primer oligomer artefact.

⁹Primer mixes 7, 10, 11, 14, 17, 18, 19, 21, 27, 30, 31, 35, 46, 53 and 65 have a tendency of giving rise to nonspecific amplifications, most pronounced in primer mix 46.

¹⁰Primer mixes 11, 14, 19, 20, 24 and 32 may yield less specific PCR products than the other B*35 primer mixes, most pronounced in primer mix 19.

¹¹Primer mix 17 may give rise to a long unspecific PCR product of approximately 600 bp. This should be disregarded when interpreting the typing results.

¹²Primer mix 46 may weakly amplify the B*73:01 and C*05:03 alleles. This primer mix may also give rise to nonspecific amplifications.

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

¹³Primer mixes 45 and 46: For many HLA-B alleles fourth exon nucleotide sequences are not available. We assume that unknown sequences in the fourth exon are conserved within allelic groups.

¹⁴Primer mix 7: Specific PCR fragment of 160 bp in the B*35:84, 35:90 and 35:150 and in the B*13:36, 27:23, 38:16, 40:08, 40:25, 40:68, 40:106, 44:12, 51:103 and 73:01^w-73:02^w alleles. Specific PCR fragment of 330 bp in the B*35:23 and in the B*15:06, 15:27:01-15:27:03, 15:84, 15:109, 15:195, 18:27, 40:100, 44:10, 47:01:01-47:03, 47:06-47:07 and 82:01-82:02 alleles.

¹⁵Primer mix 10: Specific PCR fragment of 140 bp in the B*35:83 and the C*07:63 alleles. Specific PCR fragment of 240 bp in the B*35:07 allele. Specific PCR fragment of 310 bp in the B*35:94 allele.

¹⁶Primer mix 12: Specific PCR fragment of 95 bp in the B*35:10, 35:13, 35:16, 35:28, 35:69 and 35:80 and in the B*15:202, 18:12, 37:01:01-37:01:05, 37:01:07-37:05, 37:07, 37:09-37:25, 44:83, 49:03, 52:01:01-52:01:06, 52:03-52:15, 52:17, 52:19-52:22, 53:17 and 78:05-78:06 alleles. Specific PCR fragment of 125 bp in the B*35:92 allele.

¹⁷Primer mix 16: Specific PCR fragment of 95 bp in the B*35:19 and 35:47 and in the B*15:143, 18:48, 40:08, 40:25, 40:68, 40:106 and 44:12 alleles. Specific PCR fragment of 190 bp in the B*35:77 allele.

¹⁸Primer mix 17: Specific PCR fragment of 180 bp in the B*35:26 and in the B*14:11, 15:189 and 39:04 alleles. Specific PCR fragment of 385 bp in the B*35:36 allele.

¹⁹Primer mix 18: Specific PCR fragment of 105 bp in the B*35:20:01-35:20:02, 35:28, 35:72 and 35:95 and in the B*15:202, 18:10, 18:21, 18:26, 18:35-18:36, 18:50, 37:01:01-37:01:05, 37:01:07-37:05, 37:07-37:20, 37:22-37:25, 44:83, 49:03, 51:07:01-51:07:02, 52:01:01-52:01:06, 52:03-52:05, 52:07-52:15, 52:17-52:21, 53:09, 53:17 and 78:05-78:06 alleles. Specific PCR fragment of 180 bp in the B*35:35 and 35:93 and the B*08:37, 15:50, 15:69, 15:93, 15:186, 15:199, 18:07, 18:29, 18:33, 18:37, 18:48, 39:07, 39:43, 42:09, 46:03, 46:21:01-46:21:02, 54:09, 54:14, 55:14, 55:23, 56:18, 57:26 and 58:05 and in the C*07:123, C*15:09, C*15:19-15:20, C*15:27 and C*16:20 alleles. Specific PCR fragment of 105 and 180 bp in the B*18:01:01-18:06, 18:08, 18:09, 18:11-18:15, 18:17N-18:20, 18:22-18:25, 18:27, 18:28, 18:30-18:32, 1834, 18:38-18:47, 18:49 and 18:51-18:52, alleles.

²⁰Primer mix 22: Specific PCR fragment of 90 bp in the B*35:46 and in the B*13:36, 15:02:01-15:02:05, 15:08, 15:11:01-15:11:05, 15:13, 15:15, 15:21, 15:31, 15:44, 15:55, 15:76^w, 15:88-15:89, 15:112, 15:121, 15:139, 15:144, 15:148, 15:170, 15:189, 15:191, 15:194, 15:209N, 51:104 and 55:35^w alleles. Specific PCR fragment of 270 bp in the B*35:21, 35:24:01-35:24:02, 35:81, 35:96 and 35:109 and in the B*14:10, 18:22, 37:04:01-37:04:02, 40:28, 51:04, 51:46, 51:56, 53:02, 53:06, 57:14 and 58:09 and in the C*15:39 alleles.

²¹Primer mix 25: Specific PCR fragment of 135 bp in the B*35:27 and 35:56 and in the B*18:49, 37:05 and 78:06 alleles. Specific PCR fragment of 220 bp in the *35:09:02, 35:18, 35:31, 35:64, 35:88, 35:114 and 35:118 and in the B*07:02:01-07:02:13, 07:02:15-07:67N, 07:69-07:99, 07:101-07:117, 08:01:02, 08:01:13, 08:20, 13:21, 15:02:03, 15:03:02, 15:33, 15:78:02, 15:107, 15:116, 15:141, 15:150, 15:188, 18:21, 27:01-27:21, 27:23-27:51, 27:53-27:73, 37:02, 37:07, 37:09, 38:22, 40:01:01-40:01:13, 40:01:15-40:13, 40:14:03-40:16, 40:18-40:25, 40:27, 40:29-40:40, 40:42-40:70, 40:72:02-40:76, 40:78-40:81, 40:83-40:86, 40:88-40:94, 40:96-40:120, 40:122-40:135, 40:137-40:157, 41:08, 44:31, 46:06, 47:01:01-47:07, 48:01:01-48:01:03, 48:03:01-48:11, 48:13, 48:15-48:24, 51:01:09, 51:10, 51:24:02, 51:24:04, 54:11, 55:04, 55:08, 57:01:07, 57:13, 67:03 and 81:01-81:05 alleles. Specific PCR fragment of 135 and 220 bp in the B*27:52, 51:24:03, 51:81, 58:27, alleles.

²²Primer mix 26: Specific PCR fragment of 210 bp in the B*35:50 and 35:84 and in the B*07:08, 07:32, 07:38, 38:06-38:07, 39:58, 48:06, 51:57, 51:103 and 53:21 alleles. Specific PCR fragment of 370 bp in the B*35:42:01 and B*40:106[?] alleles. Specific PCR fragment of 210 and 370 bp in the B*18:07:01-18:07:02, 18:29, 27:23, 37:21, 40:08, 40:13 and 40:68 alleles.

²³Primer mix 27: Specific PCR fragment of 110 bp in the B*35:41 allele. Specific PCR fragment of 160 bp in the B*35:84, 35:90 and 35:150 and in the B*13:36, 27:23, 38:16, 40:08, 40:25, 40:68, 40:106, 44:12, 51:103 and 73:01^w-73:02^w alleles.

²⁴Primer mix 30: Specific PCR fragment of 115 bp in the B*35:15, 35:33 and 35:66 and in the B*07:02:13, 07:68:02, 08:40, 13:01:01-13:02:09, 13:07N-13:11, 13:13-13:21, 13:22:02-13:23, 13:25-13:40, 18:10, 18:35, 18:50, 39:47, 39:50, 40:14:01, 40:72:01, 40:77, 40:95, 40:121, 40:136, 44:16, 44:21, 44:64:01-44:64:02, 45:10, 48:12, 49:07, 51:16, 51:31, 51:34, 51:82, 51:93, 52:08, 55:09, 55:22 and 55:24 and in the C*01:10^w, C*02:02:14^w and C*07:15^w alleles. Specific PCR

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

fragment of 325 bp in the B*35:25-35:26 and 35:49 and in the B*18:37, 27:01-27:21, 27:23-27:51, 27:53-27:66N, 27:68-27:73, 40:46?, 40:93?, 44:40, 44:44, 52:16, 81:01, 81:03[?]-81:05[?] and 83:01 alleles.

²⁵Primer mix 31: Specific PCR fragment of 170 bp in the B*35:25, 35:124-35:125 and 35:142 and in the B*08:01:01-08:03, 08:05, 08:07-08:16, 08:18-08:25, 08:27-08:53, 08:55, 08:57-08:60, 08:62-08:64, 08:66, 15:29 and 15:180 alleles. Specific PCR fragment of 210 bp in the B*35:50 and 35:84 and in the B*18:29, 27:23, 40:08, 40:13, 40:68, 51:57, 51:103 and 53:21 alleles. Specific PCR fragment of 170 and 210 bp in the B*07:08, 07:32, 07:38, 18:07:01-18:07:02, 37:21, 38:06-38:07, 39:58, and 48:06 alleles.

²⁶Primer mix 33: Specific PCR fragment of 70 bp in the B*35:47 and in the B*15:46, 15:106, 40:02:04, 40:10:02 and 44:84 alleles. Specific PCR fragment of 205 bp in the B*35:45 and 35:71 and in the B*07:20, 15:14, 15:91, 15:131, 15:161, 40:132, 44:10, 44:14, 44:17, 44:31, 44:43:01-44:43:02, 44:45, 44:51, 44:62, 44:77, 44:82, 44:107, 45:09, 46:17, 51:23, 52:15, 53:22, 57:07, 58:07 and 82:01-82:03 alleles. Specific PCR fragment of 310 bp in the B*35:94 allele.

²⁷Primer mix 35: Specific PCR fragment of 240 bp in the B*35:40N allele. Specific PCR fragment of 365 bp in the B*35:68:01 and the B*08:12:03 alleles.

²⁸Primer mix 36: Specific PCR fragment of 110 bp in the B*35:79 allele. Specific PCR fragment of 180 bp in the B*35:70 and the B*58:30 alleles. Specific PCR fragment of 230 bp in the B*35:35 and the B*38:20 and 39:42 alleles.

²⁹Primer mix 41: Specific PCR fragment of 105 bp in the B*35:82, 35:85 and 35:135 and in the B*18:33, 39:19:01-39:19:02, 51:22 and 78:03 alleles. Specific PCR fragment of 130 bp in the B*35:52 and 35:92 alleles.

³⁰Primer mix 42: Specific PCR fragment of 115 bp in the B*35:97 allele. Specific PCR fragment of 155 bp in the B*35:53N allele. Specific PCR fragment of 190 bp in the B*35:91 allele. Specific PCR fragment of 250 bp in the B*35:65Q allele.

³¹Primer mix 43: Specific PCR fragment of 205 bp in the B*35:54 allele. Specific PCR fragment of 255 bp in the B*35:89 allele.

³²Primer mix 44: Specific PCR fragment of 110 bp in the B*35:78 allele.

Specific PCR fragment of 145 bp in the B*18:09, 37:10, 44:83, 51:54, 51:78, 52:20, 53:09 and 53:11-53:13 alleles. Specific PCR fragment of 300 bp in the B*35:55 allele.

³³Primer mix 51: Specific PCR fragment of 65 bp in the B*35:145N allele. Specific PCR fragment of 160 bp in the B*35:100 and 35:102 alleles.

³⁴Primer mix 52: Specific PCR fragment of 220 bp in the B*35:141 allele. Specific PCR fragment of 250 bp in the B*35:104 allele.

³⁵Primer mix 53: Specific PCR fragment of 135 bp in the B*35:103 allele. Specific PCR fragment of 220 bp in the B*35:129N allele.

³⁶Primer mix 54: Specific PCR fragment of 160 bp in the B*35:106 allele. Specific PCR fragment of 190 bp in the B*35:110 allele.

³⁷Primer mix 56: Specific PCR fragment of 140 bp in the B*35:128 allele. Specific PCR fragment of 215 bp in the B*35:107 allele.

³⁸Primer mix 57: Specific PCR fragment of 150 bp in the B*35:149 allele. Specific PCR fragment of 185 bp in the B*35:111 allele.

³⁹Primer mix 58: Specific PCR fragment of 110 bp in the B*35:139 and the C*05:30 alleles. Specific PCR fragment of 160 bp in the B*35:115 and in the B*08:37, 15:51, 15:179, 15:199, 42:09, 44:17, 45:09, 53:22 and 57:24 alleles. Specific PCR fragment of 200 bp in the B*35:112 allele.

⁴⁰Primer mix 59: Specific PCR fragment of 75 bp in the B*35:116 allele. Specific PCR fragment of 150 bp in the B*35:127 and the B*57:21 alleles.

⁴¹Primer mix 60: Specific PCR fragment of 90 bp in the B*35:121 allele. Specific PCR fragment of 135 bp in the B*35:120 allele.

⁴²Primer mix 61: Specific PCR fragment of 110 bp in the B*35:139 and the C*05:30 alleles. Specific PCR fragment of 175 bp in the B*35:122 and the B*56:29 alleles. Specific PCR fragment of 265 bp in the B*35:123 allele.

⁴³Primer mix 62: Specific PCR fragment of 140 bp in the B*35:144 allele. Specific PCR fragment of 260 bp in the B*35:132 allele.

⁴⁴Primer mix 64: Specific PCR fragment of 90 bp in the B*35:130N allele. Specific PCR fragment of 245 bp in the B*35:134N allele.

Lot No.: **07L**

Lot-specific Information

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⁴⁵Primer mix 65: Specific PCR fragment of 100 bp in the B*35:131 allele. Specific PCR fragment of 220 bp in the B*35:148 allele.

⁴⁶Primer mix 66: Specific PCR fragment of 90 bp in the B*35:133 allele. Specific PCR fragment of 115 bp in the B*35:105 and 35:113 alleles.

⁴⁷Primer mix 69: Specific PCR fragment of 95 bp in the B*35:143 and in the B*51:73 and 55:26 alleles. Specific PCR fragment of 325 bp in the B*35:147 allele.

⁴⁸Primer mix 71: Specific PCR fragment of 120 bp in the B*35:126 allele. Specific PCR fragment of 160 bp in the B*35:119 allele.

‘w’, might be weakly amplified.

‘?’, nucleotide sequence of the primer matching region not known.

INTERPRETATION TABLE																																					
HLA-B*35 SSP subtyping																																					
Amplification patterns of the B*35:01 to B*35:151 alleles																																					
	Well ⁷																																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
Length of spec.	95	150	115	280	295	85	160	280	140	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
PCR product(s)							330			310	240	140					385	180																			
												125	95																								
Length of int. pos. control ¹	800	1070	800	1070	800	1070	800	1070	800	1070	800	1070	800	800	1070	1070	1070	800	800	1070	1070	800	1070	800	1070	800	800	800	1070	800	800	1070	1070	800	1070	800	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
HLA-B allele ²																																					
*35:01:01-01-35:01:03, 35:01:05-35:01:16, 35:01:18-35:01:22, 35:42:02 ⁸	1	2	3	4																			23														
*35:01:04	1	2	3	4																			23														
*35:01:17	1	2	3	4										14									23														
*35:02:01-35:02:04	1	2			5	6																	23														
*35:03:01, 35:03:03-35:03:06, 35:98 ⁹	1	2	3					8															23														
*35:03:02	1	2						8															23														
*35:03:07	1	2	3					8						14									23														
*35:04:01-35:04:02	1	2			5	6																	23														
*35:04:03	1	2			5	6								14									23														
*35:05:01-35:05:02	1		3	4					9														23					28									
*35:06	1	2			5			8															23														
*35:07	1	2	3	4					10														23														
*35:08:01-35:08:02, 35:08:04	1	2	3	4							11																										

[illegible]

Lot No.: 07L

Lot-specific Information

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[illegible]

Lot No.: 07L

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Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
*07:100						6			9				13														28			32						
*08:01:01, 08:01:03-08:01:10, 08:01:12, 08:05, 08:08N-08:12:02, 08:13, 08:15-08:16, 08:18-08:19N, 08:22, 08:24, 08:27, 08:29-08:31, 08:33-08:34, 08:36, 08:38-08:39, 08:41-08:48, 08:50-08:52, 08:55, 08:57-08:58, 08:62-08:64						5												19												31						
*08:01:02, 08:01:13						5												19							25					31						
*08:01:11						5												19												31	32					
*08:02						5												w												31						
*08:03, 08:23, 08:66						5																								31						
*08:04, 08:17, 08:26, 08:61, 08:65, 55:20						5												19																		
*08:07			3															19												31						
*08:12:03						5												19												31				35		
*08:14																		19												31						
*08:20						5							13					19							25					31						
*08:21, 08:25						5												19									28			31						
*08:28				4														19												31						
*08:32	1					5												19												31						
*08:35				4		5												19												31						
*08:37			3	4														18	19											31						
*08:40						5												19	20											30	31					
*08:49		2																19												31						
*08:53						5							13					19												31						
*08:54, 41:05, 41:12, 42:11						5																														
*08:56						5												19													32					
*08:59						5												19												31						
*08:60						5							14					19												31						
*13:01:01-13:01:02, 13:01:04-13:01:05, 13:07N, 13:17, 13:22:02-13:23, 13:25, 13:28-13:29, 13:39		2				5		8					14	15				20						23						30						
*13:01:03		2						8					14	15										23						30						
*13:02:01-13:02:09, 13:08Q-13:09, 13:11, 13:14-13:15, 13:18-13:19, 13:27, 13:30, 13:32-13:33, 13:37-13:38, 13:40						5		8					15					20						23						30						
*13:03, 15:86, 54:02, 54:23, 55:10, 55:18, 56:23, 59:01, 59:04-59:05						5		8																23												
*13:04				4																				23					29							
*13:06, 13:12		2				5		8					14											23												
*13:10				4									15																	30						
*13:13		2				5	6						14	15				20					23							30						
*13:16						5		8					13	15				20												30						
*13:20		2				5		8					13	14	15			20												30						
*13:21						5							14					20							25					30						
*13:22:01		2				5		8					14	15				20						23												
*13:26		2	3	4									15											23						30						
*13:31						5		8					13	15				20												30						
*13:34								8					15											23						30						
*13:35			3			6							15																	30						
*13:36		2				5	7	8					14	15				20					22	23			27		30							
*14:01:01-14:04, 14:06:01-14:09, 14:12, 14:14-14:19													13																							
*14:05									9				13																							
*14:10		2											13	14									22													
*14:11													13				17	19																		
*14:13						5	6		9				13																							
*15:01:01:01-15:01:04, 15:01:06-15:01:13, 15:01:15-15:01:16, 15:01:18-15:01:20, 15:24, 15:28, 15:34-15:35, 15:38:01, 15:43, 15:54, 15:56-15:57, 15:60, 15:66, 15:71, 15:79N, 15:81-15:82, 15:87, 15:92, 15:94N, 15:96-15:97, 15:104, 15:113, 15:118, 15:120, 15:122, 15:125, 15:128, 15:135, 15:140, 15:142, 15:146-15:147, 15:149N, 15:152, 15:157, 15:159-15:160, 15:163-15:164, 15:166-15:167, 15:169, 15:171-15:172, 15:174-15:175, 15:178, 15:181N-15:183, 15:187, 15:190N, 15:192-15:193, 15:201, 15:203, 15:205-15:206				4									13														28									
*15:01:14, 15:26N, 15:32, 15:110, 15:129, 15:184				4									13																							
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36

Lot No.: **07L**

Lot-specific Information

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37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Well No.
		39							46																											*07:100
									46																											*08:01:01, 08:01:03-08:01:10, 08:01:12, 08:05, 08:08N-08:12:02, 08:13, 08:15-08:16, 08:18-08:19N, 08:22, 08:24, 08:27, 08:29-08:31, 08:33-08:34, 08:36, 08:38-08:39, 08:41-08:48, 08:50-08:52, 08:55, 08:57-08:58, 08:62-08:64
									46																										72	*08:01:02, 08:01:13
									46																											*08:01:11
									46																											*08:02
									46																											*08:03, 08:23, 08:66
									46																											*08:04, 08:17, 08:26, 08:61, 08:65, 55:20
									46																											*08:07
									46																											*08:12:03
									46																											*08:14
									46																											*08:20
									46																											*08:21, 08:25
									46																											*08:28
									46																											*08:32
									46												58														*08:35	
									46																											*08:37
									46																											*08:40
									46																											*08:49
									46																											*08:53
									46																											*08:54, 41:05, 41:12, 42:11
									46																											*08:56
									46												55														*08:59	
									46																											*08:60
37									46																											*13:01:01-13:01:02, 13:01:04-13:01:05, 13:07N, 13:17, 13:22:02-13:23, 13:25, 13:28-13:29, 13:39
37									46																											*13:01:03
37									46																											*13:02:01-13:02:09, 13:08Q-13:09, 13:11, 13:14-13:15, 13:18-13:19, 13:27, 13:30, 13:32-13:33, 13:37-13:38, 13:40
37									46																											*13:03, 15:86, 54:02, 54:23, 55:10, 55:18, 56:23, 59:01, 59:04-59:05
									46																											*13:04
37									46																											*13:06, 13:12
									46																											*13:10
									46																											*13:13
37									46																											*13:16
37									46																											*13:20
									46																											*13:21
37									46																											*13:22:01
									46																											*13:26
37		39							46																											*13:31
37									46																											*13:34
									46																											*13:35
37									46																											*13:36
									46																											*14:01:01-14:04, 14:06:01-14:09, 14:12, 14:14-14:19
									46																											*14:05
									46																											*14:10
									46																											*14:11
									46																											*14:13
		39							46																											*15:01:01:01-15:01:04, 15:01:06-15:01:13, 15:01:15-15:01:16, 15:01:18-15:01:20, 15:24, 15:28, 15:34-15:35, 15:38:01, 15:43, 15:54, 15:56-15:57, 15:60, 15:66, 15:71, 15:79N, 15:81-15:82, 15:87, 15:92, 15:94N, 15:96-15:97, 15:104, 15:113, 15:118, 15:120, 15:122, 15:125, 15:128, 15:135, 15:140, 15:142, 15:146-15:147, 15:149N, 15:152, 15:157, 15:159-15:160, 15:163-15:164, 15:166-15:167, 15:169, 15:171-15:172, 15:174-15:175, 15:178, 15:181N-15:183, 15:187, 15:190N, 15:192-15:193, 15:201, 15:203, 15:205-15:206
		39							46																											*15:01:14, 15:26N, 15:32, 15:110, 15:129, 15:184
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Well No.

Lot No.: **07L**

Lot-specific Information

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37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Well No.
									46																											*15:01:17, 15:03:01, 15:03:03, 15:18:01, 15:18:03-15:18:04, 15:23, 15:39:01, 15:64-15:65, 15:72, 15:74, 15:78:01, 15:98, 15:103, 15:108, 15:115, 15:119, 15:127, 15:132, 15:134, 15:153, 15:156, 15:158, 15:173, 15:198
									46																											*15:02:01-15:02:02, 15:02:04-15:02:05, 15:13, 15:21, 15:44, 15:89, 15:112, 15:121, 15:139, 15:170
									46																										72	*15:02:03
									46																										72	*15:03:02
		39							46																											*15:04
									46																											*15:05:01, 15:123, 15:136, 15:151, 15:176, 15:185, 15:200
									46																											*15:05:02
									46																											*15:06, 15:195
		39							46																											*15:07:01-15:07:02, 15:68, 15:126, 15:207
		39							46																											*15:08, 15:11:01-15:11:02, 15:11:04-15:11:05, 15:15, 15:148, 15:191, 15:209N
									46																											*15:09
									46																											*15:10:01-15:10:02, 15:37, 15:90, 15:99
		39							46																											*15:11:03
		39							46																											*15:12, 15:19
		39							46																											*15:14, 15:46
									46																											*15:16, 15:67, 15:95, 49:05
									46																											*15:17:01-15:17:02, 15:168, 15:177, 15:196, 15:208
									46																											*15:18:02
								45																												*15:20, 48:02:01, 58:17N
									46																											*15:25:01-15:25:03, 15:36, 15:80, 15:204
		39							46																											*15:27:01-15:27:02, 15:109
		39							46																											*15:27:03
									46																											*15:29
		39							46																											*15:30
									46																											*15:31
									46																											*15:33, 15:78:02, 15:116, 15:141
		39							46																											*15:38:02
									46																											*15:39:02
									46																											*15:40, 15:47, 15:49, 15:138
37									46																											*15:42, 55:31
		39							46																											*15:45
									46																											*15:48
		39							46																											*15:50
									46																											*15:51
									46																											*15:52, 15:114, 15:124
		39	40						46																											*15:53
									46																											*15:55
		39							46																											*15:58
									46																											*15:61
									46																											*15:62
		39							46																											*15:63
									46																											*15:69, 15:93
		39							46																											*15:70, 15:101, 46:01:01, 46:01:04-46:01:05, 46:04, 46:10, 46:14-46:16, 46:20, 46:22-46:24, 56:03
37		39							46																											*15:73
		39							46																											*15:75, 15:105, 15:111N, 15:145
		39							46																											*15:76
		39							46																											*15:77
									46																											*15:83
									46																											*15:84
		39							46																											*15:85
									46																											*15:88
									46																											*15:91
		39							46																											*15:102
									46																											*15:106
									46																											*15:107
		39							46																											*15:117
									46																											*15:131, 15:161
									46																											*15:133
		39							46																											*15:137
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Well No.

Lot No.: 07L

Lot-specific Information

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Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
*15:143				4									13	14	16													28									
*15:144		2		4									13	14							22																
*15:150					5								13												25		28										
*15:154		2		4									13	14																							
*15:155				4																									29								
*15:162, 44:41, 44:65																												28									
*15:165		2		4									13															28									
*15:179			3	4																								28									
*15:180				4									13					19										28		31							
*15:186				4														18																			
*15:188					5																				25												
*15:189				4									13				17	19			22						28			32							
*15:194		2		4									13								22																
*15:197				4									13															28									
*15:199				4									13					18																			
*15:202				4								12	13				18											28									
*18:01:01-18:02, 18:05-18:06, 18:08, 18:11, 18:13, 18:17N-18:18, 18:23N-18:25, 18:28, 18:31-18:32, 18:34, 18:38-18:40, 18:42-18:47, 18:51	1		3	4													18			21																	
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*18:12				3	4							12					18			21																	
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*18:52	1		3	4													18			21		23															
*27:01, 27:31, 27:49, 27:61															15										25					30							
*27:02-27:03, 27:05:02-27:05:15, 27:09, 27:13, 27:16-27:17, 27:29, 27:35, 27:37, 27:39, 27:45-27:46, 27:48, 27:50-27:51, 27:53, 27:55-27:60, 27:64N-27:65N, 27:70, 27:72-27:73															15								24	25						30							
*27:04:01-27:04:03, 27:66N, 27:68-27:69													13	15											25					30							
*27:06, 27:21					w								13	15											25					30							
*27:07, 27:43					5														20				24	25						30							
*27:08, 27:12, 27:26, 27:42, 27:44															15										25					30							
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*27:15, 27:63													13												25					30							
*27:18, 27:40													13	15											25					30							
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*27:20, 27:24					5								13						20						25					30							
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*27:28, 27:34, 27:38, 27:47, 27:62, 27:71																								24	25					30							
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*27:33					5														20						25					30							
*27:36																									25					30							
*27:41				4											15									24	25					30							
*27:52															15										24	25											
*27:67, 47:05															15										24	25											
*37:01:01-37:01:05, 37:01:07, 37:03N, 37:15-37:20, 37:23-37:25	2											12					18			21			24														
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19																		

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Well No.
		39						46																												*15:143
		39						46																												*15:144
								46																									72			*15:150
		39						46																												*15:154
								46																												*15:155
								46																												*15:162, 44:41, 44:65
		39						46																												*15:165
								46												58															*15:179	
		39						46																												*15:180
								46																												*15:186
								46																									72			*15:188
		39						46																												*15:189
								46																												*15:194
								46																									70			*15:197
								46												58															*15:199	
		39						46																												*15:202
								46																												*18:01:01-18:02, 18:05-18:06, 18:08, 18:11, 18:13, 18:17N-18:18, 18:23N-18:25, 18:28, 18:31-18:32, 18:34, 18:38-18:40, 18:42-18:47, 18:51
								46																												*18:03
								46																												*18:04
								46	48																										*18:07:01-18:07:02	
				44				46																											*18:09	
								46																												*18:10, 18:35, 18:50
								46																												*18:12
								46																												*18:14
								46																												*18:15, 18:30
		39						46																												*18:19
								46																												*18:20
								46																									72			*18:21
								46																												*18:22
								46																												*18:26
								46																												*18:27
								46	48																										*18:29	
			41					46																												*18:33
								46																												*18:36
								46																												*18:37
								46																												*18:41
								46																												*18:48
								46																												*18:49
								46																												*18:52
								46																										72		*27:01, 27:31, 27:49, 27:61
								46																												*27:02-27:03, 27:05:02-27:05:15, 27:09, 27:13, 27:16-27:17, 27:29, 27:35, 27:37, 27:39, 27:45-27:46, 27:48, 27:50-27:51, 27:53, 27:55-27:60, 27:64N-27:65N, 27:70, 27:72-27:73
								46																												*27:04:01-27:04:03, 27:66N, 27:68-27:69
								46																												*27:06, 27:21
								46																												*27:07, 27:43
		40						46																												*27:08, 27:12, 27:26, 27:42, 27:44
								46																												*27:10, 27:54
								46																												*27:11
								46																												*27:14
								46																												*27:15, 27:63
		40						46																												*27:18, 27:40
								46																												*27:19, 27:30
								46																												*27:20, 27:24
								46	48																											*27:23
		39						46																												*27:25
								46																												*27:27
								46																												*27:28, 27:34, 27:38, 27:47, 27:62, 27:71
								46																												*27:32
		40						46																												*27:33
								46																												*27:36
								46																												*27:41
								46																												*27:52
								46																												*27:67, 47:05
								46																												*37:01:01-37:01:05, 37:01:07, 37:03N, 37:15-37:20, 37:23-37:25
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Well No.

Lot No.: 07L

Lot-specific Information

www.olerup-ssp.com

Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
*37:01:06, 37:06		2																				21	22	23	24	25											
*37:02												12			15				18			21		24	25												
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*37:10		2										12						18			21																
*37:11, 37:14		2										12						18			21																
*37:12					5							12						18			21		24														
*37:13		2										12	13					18			21		24														
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*37:22		2										12						18					24														
*38:01:01, 38:01:03-38:05, 38:08-38:09, 38:11-38:15, 38:17-38:18, 38:21, 38:23-38:26, 39:01:01-39:01:01:02L, 39:01:03-39:02:02, 39:05:01-39:05:02, 39:08-39:13:02, 39:16, 39:20, 39:22-39:23, 39:26-39:28, 39:30-39:31, 39:38Q-39:41, 39:44-39:45, 39:48, 39:51-39:54, 39:56, 39:59-39:61, 67:01:01-67:02					5			8																													
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*38:10, 39:18, 39:35					5			8					13																								
*38:16					5		7	8																			27										
*38:19, 39:03, 39:24, 39:37					5			8	9																												
*38:20, 39:42		2			5			8					14																							36	
*38:22								8																		25											
*39:04					5			8									17	19																			
*39:06:01-39:06:02, 39:33-39:34, 39:57, 39:62					5			8																						29							
*39:07, 39:43			3	4													18																				
*39:14					5	6			9																												
*39:15, 39:49, 39:55			3					8																													
*39:17, 44:15, 44:18																																					
*39:19:01	1				5			8																													
*39:19:02	1				5			8																												32	
*39:25N, 44:20, 44:47, 44:99-44:100																																					
*39:29			3			6			9																												
*39:32					5			8																													
*39:36					5			8					13																								
*39:46					5			8																													
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*39:50					5			8							15				20															29	30		
*39:58					5			8																		26								31			
*40:01:01-40:01:13, 40:01:15-40:02:03, 40:02:05-40:02:09, 40:02:11, 40:06:01-40:07, 40:11:01-40:11:02, 40:14:03, 40:22N, 40:27, 40:29, 40:37, 40:40, 40:45, 40:49-40:50, 40:53-40:58, 40:61-40:62, 40:65-40:67, 40:69, 40:72:02, 40:74-40:75, 40:78-40:81, 40:84-40:85, 40:88, 40:90-40:91, 40:97, 40:101-40:104, 40:107-40:108, 40:111-40:116, 40:118N-40:119, 40:122-40:123, 40:126-40:128, 40:130, 40:134-40:135, 40:138-40:147, 40:150-40:156					5															20					25												
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*40:02:10, 40:09, 40:18, 40:31, 40:33, 40:42, 40:44, 40:89, 40:124:01-40:124:02, 40:131																									25												
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*40:14:01, 40:72:01, 40:136					5														20														30				
*40:15-40:16, 40:23, 40:148					5								13						20						25												
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*40:24, 40:120																							23	25													
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Well No.
									46																											*37:01:06, 37:06
									46																											*37:02
									46																											*37:04:01-37:04:02
									46																											*37:05
									46																											*37:07
									46																											*37:08
									46																											*37:09
							44		46																										*37:10	
									46																											*37:11, 37:14
									46																											*37:12
									46																											*37:13
									46		48																								*37:21	
									46																											*37:22
									46																											*38:01:01, 38:01:03-38:05, 38:08-38:09, 38:11-38:15, 38:17-38:18, 38:21, 38:23-38:26, 39:01:01-39:01:01:02L, 39:01:03-39:02:02, 39:05:01-39:05:02, 39:08-39:13:02, 39:16, 39:20, 39:22-39:23, 39:26-39:28, 39:30-39:31, 39:38Q-39:41, 39:44-39:45, 39:48, 39:51-39:54, 39:56, 39:59-39:61, 67:01:01-67:02
									46																											*38:01:02
									46																											*38:06-38:07
									46																											*38:10, 39:18, 39:35
									46																											*38:16
									46																											*38:19, 39:03, 39:24, 39:37
									46																											*38:20, 39:42
									46																											*38:22
									46																											*39:04
									46																											*39:06:01-39:06:02, 39:33-39:34, 39:57, 39:62
									46																											*39:07, 39:43
									46																											*39:14
									46																											*39:15, 39:49, 39:55
37									46																											*39:17, 44:15, 44:18
				41					46																											*39:19:01
				41					46																											*39:19:02
									46																											*39:25N, 44:20, 44:47, 44:99-44:100
									46																											*39:29
									46																											*39:32
			39						46																											*39:36
									46																											*39:46
									46																											*39:47
									46																											*39:50
									46																											*39:58
			40						46																											*40:01:01-40:01:13, 40:01:15-40:02:03, 40:02:05-40:02:09, 40:02:11, 40:06:01-40:07, 40:11:01-40:11:02, 40:14:03, 40:22N, 40:27, 40:29, 40:37, 40:40, 40:45, 40:49-40:50, 40:53-40:58, 40:61-40:62, 40:65-40:67, 40:69, 40:72:02, 40:74-40:75, 40:78-40:81, 40:84-40:85, 40:88, 40:90-40:91, 40:97, 40:101-40:104, 40:107-40:108, 40:111-40:116, 40:118N-40:119, 40:122-40:123, 40:126-40:128, 40:130, 40:134-40:135, 40:138-40:147, 40:150-40:156
			40						46																											*40:01:14, 40:14:02, 40:82
									46																											*40:02:04, 40:10:02, 44:31
			40						46																											*40:02:10, 40:09, 40:18, 40:31, 40:33, 40:42, 40:44, 40:89, 40:124:01-40:124:02, 40:131
			40						46																											*40:03, 40:20, 40:52
			40						46																											*40:04, 40:129
			40						46																											*40:05
			40						46		48																									*40:08
									46																											*40:10:01, 40:12, 40:21, 40:47, 40:76, 40:83, 40:94, 40:96, 40:110, 40:157, 48:01:01-48:01:03, 48:03:01-48:04, 48:09-48:11, 48:13, 48:16, 48:18-48:22, 48:24
									46		48																									*40:13
			40						46																											*40:14:01, 40:72:01, 40:136
			40						46																											*40:15-40:16, 40:23, 40:148
									46																											*40:19, 40:109, 40:117
			40						46																											*40:24, 40:120
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Well No.

Lot No.: 07L

Lot-specific Information

www.olerup-ssp.com

Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
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*40:30, 40:34, 40:64					5									14						20					25												
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*40:36, 40:48					5		8													20					25												
*40:38, 40:105			4		5															20					25												
*40:39, 40:133Q, 41:08					5																				25												
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*40:46, 40:93					5														20						25				?								
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*40:63, 40:92					5																				25		28										
*40:68					5	7								14		16				20			23		25	26	27			31							
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*40:149					5															20	?				25												
*40:158			4		5								13	15						20																	
*41:01-41:03:02, 41:06-41:07, 41:09, 41:11, 41:13-41:16					5																																
*41:04		3																																			
*41:10, 45:03																																					
*42:01:01-42:02, 42:04-42:05:02, 42:07-42:08, 42:12, 42:14					5																													32			
*42:06					5		8																												32		
*42:09			3	4															18																32		
*42:10					5	6		9																											32		
*42:13					5									14																					32		
*44:02:01:01-44:02:05, 44:02:07-44:02:11, 44:02:13-44:03:12, 44:07-44:08, 44:11, 44:13, 44:19N, 44:22-44:24, 44:27, 44:29-44:30, 44:32-44:35, 44:37-44:39, 44:48-44:49, 44:52N, 44:55, 44:57, 44:59-44:61N, 44:63, 44:67-44:69, 44:71-44:74, 44:80-44:81, 44:85-44:86, 44:88-44:89, 44:91-44:94, 44:96-44:98, 44:101-44:105, 44:108N-44:109, 44:111-44:115			2											14																							
*44:02:06, 44:02:12, 44:36, 44:87		2																																			
*44:04, 44:28:01-44:28:02, 44:56N		2								11				14																							
*44:05:01-44:05:02, 44:42, 44:58N, 44:63, 44:70, 44:75, 44:78		2				w								14																							
*44:06, 51:42	1	2												14						20				24													
*44:09, 44:46, 44:75		2												14																							
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*44:12		2					7							14													27										
*44:14		2			5									14																						33	
*44:16, 44:21, 44:64:01-44:64:02		2												14	15															30							
*44:17		2	3	4										14																						33	
*44:25		2				w								14											24												
*44:50, 44:95, 57:32		2												14											24												
*44:26		2												14																							
*44:40, 44:44		2												14																30							
*44:43:01		2	3	4										14																					33		
*44:43:02		2		4										14																						33	
*44:45, 44:51		2	3											14																						33	
*44:53														14																							
*44:54, 44:106								9																				28									
*44:62, 44:77		2			5	6								14																						33	
*44:66		2												14																							
*44:76		2								11			13	14																							
*44:79		2											13	14																							
*44:82		2			5	6																							28						33		
*44:83		2										12	14					18																			
*44:84		2											14																							33	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	

Lot No.: 07L

Lot-specific Information

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Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
*44:90		2											14				19																			
*44:107		2	3			6							14																			33				
*44:110		2							9				14																							
*45:01, 45:04-45:05, 45:07-45:08, 45:11, 45:13																																				
*45:02																												29								
*45:06																		19																		
*45:09			3	4																								29				33				
*45:10													15																30							
*45:12					5																															
*46:01:02, 46:02, 54:06				4									13				19									28										
*46:01:03			3	4									13				19									28					32					
*46:03				4									13				18	19													32					
*46:05				4									13													28					32					
*46:06				4													19								25						32					
*46:07N				4									13				19														32					
*46:08				4									13				19									28					32					
*46:09				4													19									28					32					
*46:11, 46:18					5			8									19				23										32					
*46:12				4					9				13				19									28					32					
*46:13:01			3	4													19									28					32					
*46:13:02-46:13:03, 46:19, 46:25-46:26				4													19									28					32					
*46:17				4									13				19									28					32	33				
*46:21:01				4													18	19													32					
*46:21:02			3	4													18	19													32					
*47:01:01-47:01:01:02, 47:06						7							15									24	25													
*47:02						7							15												25											
*47:03						7							15												25											
*47:04													15												25											
*47:07						7							15									24	25													
*48:02:02		2	3	4																			23													
*48:06					5												20								25	26				31						
*48:07					5			8									20								25											
*48:12					5	6			9				15				20													30						
*48:14				4	5				9				15				20																			
*48:17																						23			25											
*48:23				4																					25											
*49:01:01-49:02, 49:06, 49:08-49:10, 49:12-49:15													13																							
*49:03												12	13				18	20																		
*49:04			3	4									13																29							
*49:07													13	15																30						
*49:11																						23														
*50:01:01-50:02, 50:04-50:08, 50:10-50:12													13																							
*50:09													13																	29						
*51:01:01, 51:01:03-51:01:08, 51:01:10, 51:01:12-51:01:14, 51:01:16-51:01:18, 51:01:20-51:02:01, 51:02:04-51:03, 51:11N-51:12, 51:21, 51:26-51:30, 51:32-51:33, 51:35-51:36, 51:38-51:39, 51:41N, 51:48-51:52, 51:55, 51:58, 51:60, 51:65-51:72, 51:74-51:75, 51:77, 51:79-51:80, 51:83-51:84, 51:86-51:88, 51:91, 51:95-51:96, 51:98N-51:102, 51:105	1					5	6						13						20					24												
*51:01:02, 51:02:02, 51:43, 51:89	1					5	6						13											24												
*51:01:09	1					5	6						13											24	25											
*51:01:11						5	6						13						20					24												
*51:01:15, 51:14, 51:24:01	1					5							13						20					24												
*51:01:19	1				w	6							13						20					24												
*51:02:03, 51:18, 51:94	1					6							13						20					24												
*51:04, 51:46	1	2				5	6						13	14					20			22		24												
*51:05, 51:09:02	1					5	6																	24												
*51:06, 51:59	1					5	6						13						20					24			28									
*51:07:01	1					5	6						13				18		20					24												
*51:07:02	1					5	6						13				18							24												
*51:08-51:09:01, 51:19, 51:40, 51:44N, 51:53	1					5	6												20					24												
*51:10, 51:81	1					5													20					24	25											
*51:13:01-51:13:02	1					5		8					13						20					24				29								
*51:15	1					5		8																23	24											
*51:16, 51:34, 51:82	1					5	6						13	15					20					24						30						
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36

Lot No.: 07L

Lot-specific Information

www.olerup-ssp.com

[illegible]

Lot No.: 07L

Lot-specific Information

www.olerup-ssp.com

Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
*58:19		2	3	4									13										24													
*58:20					5			8															24													
*58:22		2	3	4							11											23	24													
*58:27					5													20					24	25												
*58:28		2			5	6							14									23	24													
*58:30		2	3	4																		23	24													36
*59:02					5																	23														
*59:03					5			8														23							29							
*67:03					5			8																	25											
*73:01-73:02							w																			w		29			32					
*78:01, 78:07	1				5	6							13					19																		
*78:02:01-78:02:02	1				5	6							13																							
*78:03	1				5	6							13					19														32				
*78:04	1				5	6																														
*78:05					5	6					12	13					18																			
*78:06					5	6					12	13					18								25											
*81:01					5													20	21					25					30	32						
*81:02					5													20						25							32					
*81:03					5													20	?					25				?	32							
*81:04N-81:05					5													20	?					25				?	32							
*82:01-82:02					5		7	8																										33		
*82:03					5			8																										33		
*83:01	2												14				19		21										30	32						
A*01:41									w																											
A*68:56, C*06:20, C*12:50																																				
C*01:10, C*02:02:14, C*07:15																													w							
C*01:32					w																															
C*02:03, C*04:34, C*05:12, C*07:95, C*08:39																						23														
C*03:05, C*03:25, C*03:27																											28									
C*03:28, C*03:49, C*04:80, C*05:10, C*15:08											11																								34	
C*03:90																																				
C*03:102		2																																		
C*05:30																																				
C*06:21																																				
C*07:04:01-07:04:04, C*07:11-07:12, C*07:45, C*07:101, C*07:139, C*07:142																																				
C*07:63									10																											
C*07:123			3														18																			
C*15:02:05, C*15:06:03			3																																	
C*15:09, C*15:19-15:20, C*15:27, C*16:20																	18																			
C*15:39																						22														
HLA-B allele ²																																				
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*35 subtyping.

In addition, wells number 3, 5, 7, 9, 11, 13, 14, 18, 21, 23, 25, 26, 28, 30, 34, 36, 42, 45, 47, 57, 60 and 71 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

²The B*3573 allele has been renamed to B*35:08:03.

³The B*35:122 and B*35:123 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 61.

⁴The B*35:130N and B*35:134N alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 64.

⁵The B*35:131 and B*35:148 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 65.

⁶The B*35:132 and B*35:144 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 62.

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Well No.
								45																												*58:19
								46																												*58:20
								45																												*58:22
								45																									72			*58:27
								45																												*58:28
								45																												*58:30
								46																												*59:02
								46																												*59:03
								46																										72		*67:03
																																				*73:01-73:02
								45			48																									*78:01, 78:07
								45			48																									*78:02:01-78:02:02
				41				45																												*78:03
								45			48																									*78:04
								45																												*78:05
								45																												*78:06
								46																										72		*81:01
								46																										72		*81:02
								45																											72	*81:03
								46																										72		*81:04N-81:05
37								46	47																											*82:01-82:02
37								46	47																											*82:03
								?	?																											*83:01
																																				A*01:41
																																				A*68:56, C*06:20, C*12:50
																																				C*01:10, C*02:02:14, C*07:15
																																				C*01:32
																																				C*02:03, C*04:34, C*05:12, C*07:95, C*08:39
																																				C*03:05, C*03:25, C*03:27
																																				C*03:28, C*03:49, C*04:80, C*05:10, C*15:08
																																				C*03:90
																																				C*03:102
																					58			61											C*05:30	
																																				C*06:21
																																				C*07:04:01-07:04:04, C*07:11-07:12, C*07:45, C*07:101, C*07:139, C*07:142
																																				C*07:63
																																				C*07:123
																																				C*15:02:05, C*15:06:03
																																				C*15:09, C*15:19-15:20, C*15:27, C*16:20
																																				C*15:39
																																				HLA-B allele ²
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	Well No.

⁷Primer mix 7: Specific PCR fragment of 160 bp in the B*35:84, 35:90 and 35:150 and in the B*13:36, 27:23, 38:16, 40:08, 40:25, 40:68, 40:106, 44:12, 51:103 and 73:01^w-73:02^w alleles. Specific PCR fragment of 330 bp in the B*35:23 and in the B*15:06, 15:27:01-15:27:03, 15:84, 15:109, 15:195, 18:27, 40:100, 44:10, 47:01:01-47:03, 47:06-47:07 and 82:01-82:02 alleles. Primer mix 10: Specific PCR fragment of 140 bp in the B*35:83 and the C*07:63 alleles. Specific PCR fragment of 240 bp in the B*35:07 allele. Specific PCR fragment of 310 bp in the B*35:94 allele.

Primer mix 12: Specific PCR fragment of 95 bp in the B*35:10, 35:13, 35:16, 35:28, 35:69 and 35:80 and in the B*15:202, 18:12, 37:01:01-37:01:05, 37:01:07-37:05, 37:07, 37:09-37:25, 44:83, 49:03, 52:01:01-52:01:06, 52:03-52:15, 52:17, 52:19-52:22, 53:17 and 78:05-78:06 alleles. Specific PCR fragment of 125 bp in the B*35:92 allele.

Primer mix 16: Specific PCR fragment of 95 bp in the B*35:19 and 35:47 and in the B*15:143, 18:48, 40:08, 40:25, 40:68, 40:106 and 44:12 alleles. Specific PCR fragment of 190 bp in the B*35:77 allele.

Primer mix 17: Specific PCR fragment of 180 bp in the B*35:26 and in the B*14:11, 15:189 and 39:04 alleles. Specific PCR fragment of 385 bp in the B*35:36 allele.

Primer mix 18: Specific PCR fragment of 105 bp in the B*35:20:01-35:20:02, 35:28, 35:72 and 35:95 and in the B*15:202, 18:10, 18:21, 18:26, 18:35-18:36, 18:50, 37:01:01-37:01:05, 37:01:07-37:05, 37:07-37:20, 37:22-37:25, 44:83, 49:03, 51:07:01-51:07:02, 52:01:01-52:01:06, 52:03-52:05, 52:07-52:15, 52:17-52:21, 53:09, 53:17 and 78:05-78:06 alleles. Specific PCR

Lot No.: **07L**

Lot-specific Information

www.olerup-ssp.com

fragment of 180 bp in the B*35:35 and 35:93 and the B*08:37, 15:50, 15:69, 15:93, 15:186, 15:199, 18:07, 18:29, 18:33, 18:37, 18:48, 39:07, 39:43, 42:09, 46:03, 46:21:01-46:21:02, 54:09, 54:14, 55:14, 55:23, 56:18, 57:26 and 58:05 and in the C*07:123, C*15:09, C*15:19-15:20, C*15:27 and C*16:20 alleles. Specific PCR fragment of 105 and 180 bp in the B*18:01:01-18:06, 18:08, 18:09, 18:11-18:15, 18:17N-18:20, 18:22-18:25, 18:27, 18:28, 18:30-18:32, 1834, 18:38-18:47, 18:49 and 18:51-18:52, alleles.

Primer mix 22: Specific PCR fragment of 90 bp in the B*35:46 and in the B*13:36, 15:02:01-15:02:05, 15:08, 15:11:01-15:11:05, 15:13, 15:15, 15:21, 15:31, 15:44, 15:55, 15:76^w, 15:88-15:89, 15:112, 15:121, 15:139, 15:144, 15:148, 15:170, 15:189, 15:191, 15:194, 15:209N, 51:104 and 55:35^w alleles. Specific PCR fragment of 270 bp in the B*35:21, 35:24:01-35:24:02, 35:81, 35:96 and 35:109 and in the B*14:10, 18:22, 37:04:01-37:04:02, 40:28, 51:04, 51:46, 51:56, 53:02, 53:06, 57:14 and 58:09 and in the C*15:39 alleles.

Primer mix 25: Specific PCR fragment of 135 bp in the B*35:27 and 35:56 and in the B*18:49, 37:05 and 78:06 alleles. Specific PCR fragment of 220 bp in the *35:09:02, 35:18, 35:31, 35:64, 35:88, 35:114 and 35:118 and in the B*07:02:01-07:02:13, 07:02:15-07:67N, 07:69-07:99, 07:101-07:117, 08:01:02, 08:01:13, 08:20, 13:21, 15:02:03, 15:03:02, 15:33, 15:78:02, 15:107, 15:116, 15:141, 15:150, 15:188, 18:21, 27:01-27:21, 27:23-27:51, 27:53-27:73, 37:02, 37:07, 37:09, 38:22, 40:01:01-40:01:13, 40:01:15-40:13, 40:14:03-40:16, 40:18-40:25, 40:27, 40:29-40:40, 40:42-40:70, 40:72:02-40:76, 40:78-40:81, 40:83-40:86, 40:88-40:94, 40:96-40:120, 40:122-40:135, 40:137-40:157, 41:08, 44:31, 46:06, 47:01:01:01-47:07, 48:01:01-48:01:03, 48:03:01-48:11, 48:13, 48:15-48:24, 51:01:09, 51:10, 51:24:02, 51:24:04, 54:11, 55:04, 55:08, 57:01:07, 57:13, 67:03 and 81:01-81:05 alleles. Specific PCR fragment of 135 and 220 bp in the B*27:52, 51:24:03, 51:81, 58:27, alleles.

Primer mix 26: Specific PCR fragment of 210 bp in the B*35:50 and 35:84 and in the B*07:08, 07:32, 07:38, 38:06-38:07, 39:58, 48:06, 51:57, 51:103 and 53:21 alleles. Specific PCR fragment of 370 bp in the B*35:42:01 and B*40:106[?] alleles. Specific PCR fragment of 210 and 370 bp in the B*18:07:01-18:07:02, 18:29, 27:23, 37:21, 40:08, 40:13 and 40:68 alleles.

Primer mix 27: Specific PCR fragment of 110 bp in the B*35:41 allele. Specific PCR fragment of 160 bp in the B*35:84, 35:90 and 35:150 and in the B*13:36, 27:23, 38:16, 40:08, 40:25, 40:68, 40:106, 44:12, 51:103 and 73:01^w-73:02^w alleles.

Primer mix 30: Specific PCR fragment of 115 bp in the B*35:15, 35:33 and 35:66 and in the B*07:02:13, 07:68:02, 08:40, 13:01:01-13:02:09, 13:07N-13:11, 13:13-13:21, 13:22:02-13:23, 13:25-13:40, 18:10, 18:35, 18:50, 39:47, 39:50, 40:14:01, 40:72:01, 40:77, 40:95, 40:121, 40:136, 44:16, 44:21, 44:64:01-44:64:02, 45:10, 48:12, 49:07, 51:16, 51:31, 51:34, 51:82, 51:93, 52:08, 55:09, 55:22 and 55:24 and in the C*01:10^w, C*02:02:14^w and C*07:15^w alleles. Specific PCR fragment of 325 bp in the B*35:25-35:26 and 35:49 and in the B*18:37, 27:01-27:21, 27:23-27:51, 27:53-27:66N, 27:68-27:73, 40:46[?], 40:93[?], 44:40, 44:44, 52:16, 81:01, 81:03[?]-81:05[?] and 83:01 alleles.

Primer mix 31: Specific PCR fragment of 170 bp in the B*35:25, 35:124-35:125 and 35:142 and in the B*08:01:01-08:03, 08:05, 08:07-08:16, 08:18-08:25, 08:27-08:53, 08:55, 08:57-08:60, 08:62-08:64, 08:66, 15:29 and 15:180 alleles. Specific PCR fragment of 210 bp in the B*35:50 and 35:84 and in the B*18:29, 27:23, 40:08, 40:13, 40:68, 51:57, 51:103 and 53:21 alleles. Specific PCR fragment of 170 and 210 bp in the B*07:08, 07:32, 07:38, 18:07:01-18:07:02, 37:21, 38:06-38:07, 39:58, and 48:06 alleles.

Primer mix 33: Specific PCR fragment of 70 bp in the B*35:47 and in the B*15:46, 15:106, 40:02:04, 40:10:02 and 44:84 alleles. Specific PCR fragment of 205 bp in the B*35:45 and 35:71 and in the B*07:20, 15:14, 15:91, 15:131, 15:161, 40:132, 44:10, 44:14, 44:17, 44:31, 44:43:01-44:43:02, 44:45, 44:51, 44:62, 44:77, 44:82, 44:107, 45:09, 46:17, 51:23, 52:15, 53:22, 57:07, 58:07 and 82:01-82:03 alleles. Specific PCR fragment of 310 bp in the B*35:94 allele.

Primer mix 35: Specific PCR fragment of 240 bp in the B*35:40N allele. Specific PCR fragment of 365 bp in the B*35:68:01 and the B*08:12:03 alleles.

Primer mix 36: Specific PCR fragment of 110 bp in the B*35:79 allele. Specific PCR fragment of 180 bp in the B*35:70 and the B*58:30 alleles. Specific PCR fragment of 230 bp in the B*35:35 and the B*38:20 and 39:42 alleles.

Primer mix 41: Specific PCR fragment of 105 bp in the B*35:82, 35:85 and 35:135 and in the B*18:33, 39:19:01-39:19:02, 51:22 and 78:03 alleles. Specific PCR fragment of 130 bp in the B*35:52 and 35:92 alleles.

Lot No.: **07L**

Lot-specific Information

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Primer mix 42: Specific PCR fragment of 115 bp in the B*35:97 allele. Specific PCR fragment of 155 bp in the B*35:53N allele. Specific PCR fragment of 190 bp in the B*35:91 allele. Specific PCR fragment of 250 bp in the B*35:65Q allele.

Primer mix 43: Specific PCR fragment of 205 bp in the B*35:54 allele. Specific PCR fragment of 255 bp in the B*35:89 allele.

Primer mix 44: Specific PCR fragment of 110 bp in the B*35:78 allele.

Specific PCR fragment of 145 bp in the B*18:09, 37:10, 44:83, 51:54, 51:78, 52:20, 53:09 and 53:11-53:13 alleles. Specific PCR fragment of 300 bp in the B*35:55 allele.

Primer mix 51: Specific PCR fragment of 65 bp in the B*35:145N allele. Specific PCR fragment of 160 bp in the B*35:100 and 35:102 alleles.

Primer mix 52: Specific PCR fragment of 220 bp in the B*35:141 allele. Specific PCR fragment of 250 bp in the B*35:104 allele.

Primer mix 53: Specific PCR fragment of 135 bp in the B*35:103 allele. Specific PCR fragment of 220 bp in the B*35:129N allele.

Primer mix 54: Specific PCR fragment of 160 bp in the B*35:106 allele. Specific PCR fragment of 190 bp in the B*35:110 allele.

Primer mix 56: Specific PCR fragment of 140 bp in the B*35:128 allele. Specific PCR fragment of 215 bp in the B*35:107 allele.

Primer mix 57: Specific PCR fragment of 150 bp in the B*35:149 allele. Specific PCR fragment of 185 bp in the B*35:111 allele.

Primer mix 58: Specific PCR fragment of 110 bp in the B*35:139 and the C*05:30 alleles. Specific PCR fragment of 160 bp in the B*35:115 and in the B*08:37, 15:51, 15:179, 15:199, 42:09, 44:17, 45:09, 53:22 and 57:24 alleles. Specific PCR fragment of 200 bp in the B*35:112 allele.

Primer mix 59: Specific PCR fragment of 75 bp in the B*35:116 allele. Specific PCR fragment of 150 bp in the B*35:127 and the B*57:21 alleles.

Primer mix 60: Specific PCR fragment of 90 bp in the B*35:121 allele. Specific PCR fragment of 135 bp in the B*35:120 allele.

Primer mix 61: Specific PCR fragment of 110 bp in the B*35:139 and the C*05:30 alleles. Specific PCR fragment of 175 bp in the B*35:122 and the B*56:29 alleles. Specific PCR fragment of 265 bp in the B*35:123 allele.

Primer mix 62: Specific PCR fragment of 140 bp in the B*35:144 allele. Specific PCR fragment of 260 bp in the B*35:132 allele.

Primer mix 64: Specific PCR fragment of 90 bp in the B*35:130N allele.

Specific PCR fragment of 245 bp in the B*35:134N allele.

Primer mix 65: Specific PCR fragment of 100 bp in the B*35:131 allele. Specific PCR fragment of 220 bp in the B*35:148 allele.

Primer mix 66: Specific PCR fragment of 90 bp in the B*35:133 allele. Specific PCR fragment of 115 bp in the B*35:105 and 35:113 alleles.

Primer mix 69: Specific PCR fragment of 95 bp in the B*35:143 and in the B*51:73 and 55:26 alleles. Specific PCR fragment of 325 bp in the B*35:147 allele.

Primer mix 71: Specific PCR fragment of 120 bp in the B*35:126 allele. Specific PCR fragment of 160 bp in the B*35:119 allele.

⁸This lot of the HLA-B*35 kit does not separate the B*35:01:01:01-35:01:03, 35:01:05-35:01:16 and 35:01:18-35:01:22 and the B*35:42:02 alleles.

⁹This lot of the B*35 subtyping kit cannot distinguish the B*35:98 and the B*35:03:01 and 35:03:03-35:03:06 alleles.

‘w’, might be weakly amplified.

‘?’ , nucleotide sequence of the primer matching region not known.

Primers

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
5'-primer(s) ¹	206 5'-gAC 3'	355 5'-TCA 3'	412 5'-ATg 3'	419 5'-gTC 3'	397 5'-gCT 3'	419 5'-gTA 3'	142 5'-TCA 3'	419 5'-gTT 3'	363 5'-AgC 3'	49 5'-CAg 3'	355 5'-TCA 3'	206 5'-gAC 3'
					412 5'-ATA 3'		368 5'-gTT 3'	419 5'-gTT 3'	363 5'-AgC 3'	388 5'-CCT 3'		
										742 5'-ACA 3'		
3'-primer(s) ²	259 5'-gTT 3'	463 5'-gCT 3'	486 5'-gCg 3'	3 rd I 5'-TAT 3'	3 rd I 5'-TAT 3'	463 5'-gCT 3'	259 5'-gTT 3'	3 rd I 5'-TAT 3'	463 5'-gCT 3'	119 5'-ggA 3'	538 5'-CCg 3'	259 5'-CTC 3'
							3 rd I 5'-TAT 3'			486 5'-gCg 3'		289 5'-AgC 3'
										916 5'-gAT 3'		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15	16	17	18	19	20	21	22	23	24
5'-primer(s) ¹	527 5'-TgA 3'	355 5'-TCA 3'	463 5'-TgA 3'	112 5'-CCg 3'	106 5'-CCA 3'	206 5'-gAC 3'	106 5'-CCA 3'	141 5'-ATT 3'	49 5'-CAg 3'	209 5'-ggC 3'	379 5'-ACC 3'	49 5'-CAg 3'
				206 5'-gAA 3'	320 5'-CgA 3'	419 5'-gTC 3'		412 5'-ATA 3'		355 5'-TCA 3'		
3'-primer(s) ²	3 rd I 5'-TAT 3'	387 5'-TCC 3'	559 5'-CTC 3'	259 5'-gTT 3'	246 5'-TAT 3'	272 5'-Tgg 3'	292 5'-gTC 3'	309 5'-ATC 3'	142 5'-TgA 3'	259 5'-gTT 3'	527 5'-CCA 3'	301 5'-gTC 3'
					419 5'-CgA 3'	559 5'-CgT 3'	299 5'-TCA 3'	559 5'-CTC 3'		583 5'-gTg 3'	527 5'-CCA 3'	309 5'-ATC 3'
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

Well No.	25	26	27	28	29	30	31	32	33	34	35	36
5'-primer(s) ¹	206 5'-gAC 3'	72 5'-gCT 3'	142 5'-TCA 3'	369 5'-TAC 3'	357 5'-Tgg 3'	49 5'-CAg 3'	103 5'-CCT 3'	165 5'-ACC 3'	165 5'-ACC 3'	368 5'-gTC 3'	272 5'-CTT 3'	355 5'-TCA 3'
	477 5'-gCC 3'	103 5'-CCT 3'	355 5'-TCA 3'			486 5'-ACC 3'	103 5'-CCT 3'		409 5'-ggC 3'		808 5'-CgC 3'	635 5'-ACg 3'
		103 5'-CCT 3'					142 5'-TCT 3'		742 5'-ACA 3'			704 5'-TgA 3'
3'-primer(s) ²	302 5'-ggT 3'	272 5'-TgA 3'	259 5'-gTT 3'	559 5'-CAg 3'	419 5'-Cgg 3'	204 5'-TCT 3'	272 5'-TgA 3'	292 5'-gTC 3'	193 5'-CgT 3'	559 5'-CAg 3'	353 5'-ggg 3'	544 5'-ggT 3'
	3 rd I 5'-TAT 3'		425 5'-CgC 3'		419 5'-CgA 3'	559 5'-CTC 3'			572 5'-gCg 3'		916 5'-gAT 3'	774 5'-ggT 3'
									916 5'-gAT 3'			
Well No.	25	26	27	28	29	30	31	32	33	34	35	36

Well No.	37	38	39	40	41	42	43	44	45	46	47	48
5'-primer(s) ¹	420 5'-TTA 3'	397 5'-gCT 3'	463 5'-TgA 3'	167 5'-gCT 3'	206 5'-gAC 3'	106 5'-CCA 3'	106 5'-CCg 3'	206 5'-gAC 3'	652 5'-CCg 3'	652 5'-CCA 3'	419 5'-gTT 3'	49 5'-CAg 3'
						355 5'-TCA 3'	363 5'-AgC 3'	355 5'-TCA 3'				
							363 5'-AgC 3'					
3'-primer(s) ²	463 5'-gCT 3'	3 rd I 5'-TAT 3'	538 5'-CCA 3'	311 5'-ggT 3'	272 5'-TgC 3'	181 5'-gTg 3'	272 5'-TgA 3'	277 5'-ggC 3'	774 5'-ggT 3'	774 5'-ggT 3'	538 5'-gTC 3'	272 5'-TgA 3'
					289 5'-AgC 3'	471 5'-TC 3'	577 5'-gCA 3'	309 5'-gTg 3'				
					301 5'-gCA 3'	505 5'-gCT 3'		614 5'-CgT 3'				
						564 5'-ACC 3'						
Well No.	37	38	39	40	41	42	43	44	45	46	47	48

Lot No.: **07L**

Lot-specific Information

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Well No.	49	50	51	52	53	54	55	56	57	58	59	60
5'-primer(s) ¹	272	206	190	355	106	206	106	136	106	106	352	367
	5'-CTT 3'	5'-gAC 3'	5'-ACT 3'	5'-TCA 3'	5'-CCA 3'	5'-gAC 3'	5'-CCA 3'	5'-gCC 3'	5'-CCA 3'	5'-CCA 3'	5'-ACg 3'	5'-TgC 3'
			199			355		211		419	431	412
			5'-gTg 3'			5'-TCA 3'		5'-AgT 3'		5'-gTC 3'	5'-CgA 3'	5'-ATC 3'
			533									
			5'-ggg 3'									
3'-primer(s) ²	369	343	311	532	200	325	178	311	214	176	463	463
	5'-CCg 3'	5'-g 3'	5'-ggT 3'	5'-CTg 3'	5'-TCA 3'	5'-gTg 3'	5'-gAg 3'	5'-ggT 3'	5'-CCC 3'	5'-ACT 3'	5'-gCT 3'	5'-gCT 3'
		343	559	565	286	506	180		250	267		
		5'-T 3'	5'-CAg 3'	5'-CAg 3'	5'-CTA 3'	5'-TgT 3'	5'-TCC 3'		5'-CCC 3'	5'-ATA 3'		
										538		
										5'-gTC 3'		
Well No.	49	50	51	52	53	54	55	56	57	58	59	60

Well No.	61	62	63	64	65	66	67	68	69	70	71	72
5'-primer(s) ¹	106	355	397	106	134	387	106	167	106	363	344	477
	5'-CCA 3'	5'-TCA 3'	5'-gCT 3'	5'-CCA 3'	5'-CCT 3'	5'-TCC 3'	5'-CCA 3'	5'-gCT 3'	5'-CCA 3'	5'-AgT 3'	5'-A 3'	5'-gCC 3'
				804	406	413		463	727		383	
				5'-TgA 3'	5'-gCC 3'	5'-TgT 3'		5'-TgA 3'	5'-Agg 3'		5'-ggC 3'	
3'-primer(s) ²	176	456	419	153	311	463	171	206	160	463	463	3 rd I
	5'-ACT 3'	5'-TCg 3'	5'-Cgg 3'	5'-ACT 3'	5'-ggT 3'	5'-gCT 3'	5'-ACC 3'	5'-CCg 3'	5'-gTT 3'	5'-gCT 3'	5'-gCT 3'	5'-TAT 3'
	239	575		916	463			524	916			
	5'-gCT 3'	5'-ggg 3'		5'-gAT 3'	5'-gCT 3'			5'-CAT 3'	5'-gAT 3'			
	332											
	5'-TCC 3'											
Well No.	61	62	63	64	65	66	67	68	69	70	71	72

¹The nucleotide position, in the 1st, 2nd, 3rd or 4th exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

²The nucleotide position, in the 2nd, 3rd or 4th exon or the 3rd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

[illegible]

CELL LINE VALIDATION SHEET									
HLA-B*35 SSP subtyping									
						Well			
						65	66	67	68
					Prod. No.:	69	70	71	72
						201182465	201182466	201182467	201182468
						201182469	201182470	201182471	201182472
	IHWC cell line	B*							
1	9001 SA	*07:02				-	-	-	+
2	9280 LK707	*52:01	*73:01			-	-	-	-
3	9011 E4181324	*52:01				-	-	-	-
4	9275 GU373	*15:10	*53:01			-	-	-	-
5	9009 KAS011	*37:01				-	-	-	-
6	9353 SM	*39:01	*51:01			-	-	-	-
7	9020 QBL	*18:01				-	-	-	-
8	9025 DEU	*35:01				-	-	-	-
9	9026 YAR	*38:01				-	-	-	-
10	9107 LKT3	*54:01				-	-	-	-
11	9051 PITOUT	*44:03				-	-	-	-
12	9052 DBB	*57:01				-	-	-	-
13	9004 JESTHOM	*27:05				-	-	-	+
14	9071 OLGA	*15:01	*15:20			-	-	-	-
15	9075 DKB	*40:01				-	-	-	+
16	9037 SWEIG007	*40:02				-	-	-	+
17	9282 CTM3953540	*08:01	*55:01			-	-	-	-
18	9257 32367	*14:01	*56:01			-	-	-	-
19	9038 BM16	*18:01				-	-	-	-
20	9059 SLE005	*40:01				-	-	-	+
21	9064 AMALA	*15:01				-	-	-	-
22	9056 KOSE	*35:03				-	-	-	-
23	9124 IHL	*40:02	*56:02			-	-	-	+
24	9035 JBUSH	*38:01				-	-	-	-
25	9049 IBW9	*14:02				-	-	-	-
26	9285 WT49	*58:01				-	-	-	-
27	9191 CH1007	*07:05	*51:01			-	-	-	+
28	9320 BEL5GB	*44:02	*44:03			-	-	-	-
29	9050 MOU	*44:03				-	-	-	-
30	9021 RSH	*42:01				-	-	-	-
31	9019 DUCAF	*18:01				-	-	-	-
32	9297 HAG	*41:02				-	-	-	-
33	9098 MT14B	*40:01				-	-	-	+
34	9104 DHIF	*38:01				-	-	-	-
35	9302 SSTO	*44:02				-	-	-	-
36	9024 KT17	*15:01	*35:01			-	-	-	-
37	9065 HHKB	*07:02				-	-	-	+
38	9099 LZL	*15:01				-	-	-	-
39	9315 CML	*08:01	*27:05			-	-	-	+
40	9134 WHONP199	*13:02	*46:01			-	-	-	-
41	9055 H0301	*14:02				-	-	-	-
42	9066 TAB089	*46:01				-	-	-	-
43	9076 T7526	*46:01				-	-	-	-
44	9057 TEM	*38:01				-	-	-	-
45	9239 SHJO	*42:01	*50:01			-	-	-	-
46	9013 SCHU	*07:02				-	-	-	+
47	9045 TUBO	*51:01				-	-	-	-
48	9303 TER-ND	*35:01	*44:03			-	-	-	-

CERTIFICATE OF ANALYSIS**Olerup SSP® HLA-B*35 SSP**

Product number:

101.522-24/03 – including *Taq* pol.

Lot number:

07L

Expiry date:

2013-September -01

Number of tests:

24 tests – Product No. 101.522-24

3 tests – Product No. 101.522-03

Number of wells per test:

72

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2009-667-01	25	2011-824-25	49	2011-824-49
2	2007-354-02	26	2009-667-26	50	2011-824-50
3	2007-354-03	27	2009-667-27	51	2011-824-51
4	2007-354-04	28	2007-354-28	52	2011-824-52
5	2011-824-05	29	2007-354-29	53	2011-824-53
6	2007-354-06	30	2007-354-30	54	2011-824-54
7	2009-667-07	31	2011-824-31	55	2011-824-55
8	2007-354-08	32	2007-354-32	56	2011-824-56
9	2008-491-09	33	2009-667-33	57	2011-824-57
10	2009-667-10	34	2007-354-34	58	2011-824-58
11	2007-354-11	35	2007-354-35	59	2011-824-59
12	2009-667-12	36	2008-491-36	60	2011-824-60
13	2007-354-13	37	2007-354-37	61	2011-824-61
14	2007-354-14	38	2011-824-38	62	2011-824-62
15	2007-354-15	39	2007-354-39	63	2011-824-63
16	2007-354-16	40	2007-354-40	64	2011-824-64
17	2007-354-17	41	2009-667-41	65	2011-824-65
18	2009-667-18	42	2009-667-42	66	2011-824-66
19	2007-354-19	43	2009-667-43	67	2011-824-67
20	2007-354-20	44	2008-491-44	68	2011-824-68
21	2007-354-21	45	2007-354-45	69	2011-824-69
22	2007-354-22	46	2011-824-46	70	2011-824-70
23	2007-354-23	47	2009-667-47	71	2011-824-71
24	2009-667-24	48	2007-354-48	72	2011-824-72

The specificity of each primer solution of the kit has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 10, 33, 35, 36, 41 to 44 and 50 to 71 were available.

The specificities of the primers in primer solutions 10, 33, 35, 36, 41, 43, 44, 50, 58, 61, 63, 64, 68 and 69 were tested by separately adding one or two additional 5'-primers, respectively one or two additional 3'-primers.

In primer solutions 42, 52 to 55, 57, 62 and 67 it was only possible to test the 5'-primers, the 3'-primers were not possible to test.

In primer solutions 51, 56, 59, 60, 65, 66, 70 and 71 it was only possible to test the 3'-primers, the 5'-primers were not possible to test.

In primer solutions 10, 16, 33, 35, 36, 43, 64 and 69 one or two of the 5'-primers were not possible to test, and in primer solutions 10, 12, 35, 41, 43, 44, 50, 58, 61, 64 and 69 one or two of the 3'-primers were not possible to test.

Additional primers in primer solutions 22, 26 and 31 were tested by separately adding one 5'-primer and/or one 3'-primer.

Results: No false positive or false negative amplifications were obtained.

Date of approval: 2011-August-18

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*35
Product number: 101.522-24/03
Lot number: 07L

Intended use: HLA-B*35 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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SE-112 51 Stockholm, Sweden
Phone: +46-8-717 88 27
Fax: +46-8-717 88 18

We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2008 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, conformity assessed using Annex IV, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at *Olerup* SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom.
(Notified Body number: 0088.)

Stockholm, Sweden
2011-August-18

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

Lot No.: **07L**

Lot-specific Information

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