

101.513-24/04– including *Taq* pol., IFU-01
101.513-24u/04u – without *Taq* pol., IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **29X**

Lot-specific information

Olerup SSP® HLA-B*08

Product number:	101.513-24/04 – including <i>Taq</i> pol. 101.513-24u/04u – without <i>Taq</i> pol.
Lot number:	29X
Expiry date:	2017-April-01
Number of tests:	24 tests – Product No. 101.513-24/24u 4 tests – Product No. 101.513-04/04u
Number of wells per test:	47+1
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. 29X.

Complete product documentation consists of generic Instructions for Use (IFU), lot specific Product Insert, Worksheet and Certificate.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP® HLA-B*08 LOT (35S)

The HLA-B*08 kit is updated for new alleles to enable separation of:

- Confirmed¹ alleles as listed in the IMGT/HLA database
- Polymorphisms in exons outside of the region encoding the peptide binding domain
- Null and Alternatively expressed alleles

A well containing Negative Control primer pairs has been added.

The format of the Product Insert and Worksheet have been changed.

¹As described in section Uniquely Identified Alleles.

The HLA-B*08 primer set, specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP®* HLA-B*08 lot was made (**Lot No. 35S**). The kit design is based on IMGT/HLA database 3.17.0.

As of lot series V, the Specificity Table is included in the lot-specific Product Insert, and the Interpretation Table is included in the Worksheet.

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

Well	5'-primer	3'-primer	rationale
4	Added	Added	Primer pair added for the B*08:113 allele.
9	Added	-	5'-primer added for the B*08:71 allele.
22	-	Added	3'-primer added for the B*08:109 allele.
24	Added	-	5'-primer added for the B*08:101 allele.
25	Added	-	5'-primer added for the B*08:71 allele.
28	Added	Added	Primer pair added from well 46.
30	Moved, added	Moved, added	Primer pair moved to well 36, primer pair added from well 48.
32	Added	Added	5'-primer added for the B*08:01:23, 3'-primer added for the B*08:01:25 allele.
34	Added	Added	Primer pair added for the B*08:84 allele.
35	Added	Added	Primer pair added for the B*08:113 allele.
36	Added	-	5'-primer added from well 30.
37	Added	-	5'-primer added for the B*08:105 allele.
38	Added	-	5'-primer added for the B*08:105 allele.
41	Added	Added	Primer pair added for the B*08:108 allele.
46	Moved, added	Moved, added	Primer pair moved to well 28, primer pair added for the B*08:104 allele.
47	-	Added	3'-primer added for the allelic resolution of B*08:78 and B*08:03 alleles.
48	Moved	Moved	Primer pair moved to well 30, negative control.

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PRODUCT DESCRIPTION

HLA-B*08 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*08:01 to B*08:134 alleles.

PLATE LAYOUT

Each test consists of 48 PCR reactions in a 48 well cut PCR plate.

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	NC

The 48 well cut PCR plate is marked with 'HLA-B*08' in silver/gray ink.

Well No. 1 is marked with the Lot No. '29X'.

Wells 1 to 47– HLA-B*08 high resolution primers.

Well 48 – Negative Control (NC).

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.

Please note: When removing each 48 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Due to the sharing of sequence motifs between HLA-B alleles, non-HLA-B*08 alleles will be amplified by primer mixes 1 to 21, 23 to 26, 29, 32 to 34, 36, 37, 40, 41, 43 to 45 and 47. In addition, a few HLA-C alleles will be amplified by primer mixes 3, 7, 8, 15, 23, 25, 29 and 43.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*08 alleles, i.e. **B*08:01 to B*08:134**, recognized by the HLA Nomenclature Committee in July 2014^{1,2} will be amplified by the primers in the HLA-B*08 subtyping kit³.

The HLA-B*08 kit enables separation of the confirmed HLA-B*08 alleles as listed in the IMGT/HLA database. An HLA allele is listed as confirmed by IMGT/HLA if it has been sequenced by more than a single laboratory or from multiple sources. Current allele confirmation status for HLA-B*08 alleles is listed below.

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The HLA-B*08 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.

The following HLA-B*08 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
B*08:11, 08:76	13	B*08:45, 08:59:01-08:59:02	34
B*08:19N, 08:109	22	B*08:51, 08:57	35
B*08:21, 08:47	23	B*08:58, 08:61	38
B*08:33, 08:53:01-08:53:02	36	B*08:68, 08:69	45
B*08:41, 08:48	37		

The HLA-B*08 subtyping primer set cannot distinguish the silent mutations in the B*08:01:01-08:01:30 alleles, the B*08:12:01-08:12:03 alleles, the B*08:26:01-08:26:02 alleles, the B*08:53:01-08:53:02 alleles, the B*08:56:01-08:56:02 or the 08:59:01-08:59:02 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2014-July-25, release 3.17.0, www.ebi.ac.uk/imgt/hla.

²Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page <http://hla.alleles.org/alleles/deleted.html>.

³The HLA-B*08 primer set cannot separate the B*08:94 and the B*35:87 alleles. These alleles can be distinguished by the HLA-B low resolution kit and/or the HLA-B*35 high resolution kit.

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ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
B*08:01:01	Confirmed	B*08:21	Unconfirmed	B*08:67N	Unconfirmed	B*08:117	Unconfirmed
B*08:01:02	Confirmed	B*08:22	Unconfirmed	B*08:68	Unconfirmed	B*08:118	Unconfirmed
B*08:01:03	Unconfirmed	B*08:23	Confirmed	B*08:69	Unconfirmed	B*08:119	Unconfirmed
B*08:01:04	Confirmed	B*08:24	Confirmed	B*08:70	Confirmed	B*08:120	Unconfirmed
B*08:01:05	Confirmed	B*08:25	Confirmed	B*08:71	Confirmed	B*08:121	Unconfirmed
B*08:01:06	Unconfirmed	B*08:26:01	Unconfirmed	B*08:72N	Unconfirmed	B*08:122	Unconfirmed
B*08:01:07	Confirmed	B*08:26:02	Unconfirmed	B*08:73	Unconfirmed	B*08:123	Unconfirmed
B*08:01:08	Confirmed	B*08:27	Unconfirmed	B*08:74	Unconfirmed	B*08:124	Unconfirmed
B*08:01:09	Unconfirmed	B*08:28	Confirmed	B*08:75	Unconfirmed	B*08:125	Unconfirmed
B*08:01:10	Confirmed	B*08:29	Unconfirmed	B*08:76	Unconfirmed	B*08:126	Unconfirmed
B*08:01:11	Unconfirmed	B*08:30N	Unconfirmed	B*08:77	Unconfirmed	B*08:127	Unconfirmed
B*08:01:12	Unconfirmed	B*08:31	Unconfirmed	B*08:78	Confirmed	B*08:128	Unconfirmed
B*08:01:13	Confirmed	B*08:32	Unconfirmed	B*08:79	Unconfirmed	B*08:129	Unconfirmed
B*08:01:14	Unconfirmed	B*08:33	Unconfirmed	B*08:80	Unconfirmed	B*08:130	Unconfirmed
B*08:01:15	Confirmed	B*08:34	Unconfirmed	B*08:81	Unconfirmed	B*08:131	Unconfirmed
B*08:01:16	Unconfirmed	B*08:35	Confirmed	B*08:82N	Unconfirmed	B*08:132	Unconfirmed
B*08:01:17	Confirmed	B*08:36	Unconfirmed	B*08:83	Unconfirmed	B*08:133	Unconfirmed
B*08:01:18	Unconfirmed	B*08:37	Confirmed	B*08:84	Confirmed	B*08:134	Unconfirmed
B*08:01:19	Unconfirmed	B*08:38	Unconfirmed	B*08:85	Unconfirmed		
B*08:01:20	Unconfirmed	B*08:39	Confirmed	B*08:86N	Unconfirmed		
B*08:01:21	Confirmed	B*08:40	Confirmed	B*08:87	Unconfirmed		
B*08:01:22	Unconfirmed	B*08:41	Confirmed	B*08:88	Unconfirmed		
B*08:01:23	Unconfirmed	B*08:42	Unconfirmed	B*08:89	Unconfirmed		
B*08:01:24	Unconfirmed	B*08:43	Unconfirmed	B*08:90	Unconfirmed		
B*08:01:25	Unconfirmed	B*08:44	Unconfirmed	B*08:91	Unconfirmed		
B*08:01:26	Unconfirmed	B*08:45	Unconfirmed	B*08:92	Unconfirmed		
B*08:01:27	Unconfirmed	B*08:46	Unconfirmed	B*08:93	Unconfirmed		
B*08:01:28	Unconfirmed	B*08:47	Confirmed	B*08:94	Confirmed		
B*08:01:29	Confirmed	B*08:48	Unconfirmed	B*08:95	Unconfirmed		
B*08:01:30	Unconfirmed	B*08:49	Unconfirmed	B*08:96	Unconfirmed		
B*08:02	Confirmed	B*08:50	Unconfirmed	B*08:97	Unconfirmed		
B*08:03	Confirmed	B*08:51	Unconfirmed	B*08:98	Unconfirmed		
B*08:04	Confirmed	B*08:52	Confirmed	B*08:99	Unconfirmed		
B*08:05	Unconfirmed	B*08:53:01	Confirmed	B*08:100	Confirmed		
B*08:07	Unconfirmed	B*08:53:02	Unconfirmed	B*08:101	Confirmed		
B*08:08N	Confirmed	B*08:54	Unconfirmed	B*08:102	Unconfirmed		
B*08:09	Confirmed	B*08:55	Unconfirmed	B*08:103	Unconfirmed		
B*08:10	Unconfirmed	B*08:56:01	Confirmed	B*08:104	Confirmed		
B*08:11	Unconfirmed	B*08:56:02	Confirmed	B*08:105	Confirmed		
B*08:12:01	Confirmed	B*08:57	Unconfirmed	B*08:106	Unconfirmed		
B*08:12:02	Unconfirmed	B*08:58	Unconfirmed	B*08:107	Unconfirmed		
B*08:12:03	Confirmed	B*08:59:01	Confirmed	B*08:108	Confirmed		
B*08:13	Confirmed	B*08:59:02	Unconfirmed	B*08:109	Unconfirmed		
B*08:14	Unconfirmed	B*08:60	Unconfirmed	B*08:110	Confirmed		
B*08:15	Unconfirmed	B*08:61	Unconfirmed	B*08:111	Unconfirmed		
B*08:16	Confirmed	B*08:62	Confirmed	B*08:112	Unconfirmed		
B*08:17	Unconfirmed	B*08:63	Confirmed	B*08:113	Confirmed		
B*08:18	Confirmed	B*08:64	Unconfirmed	B*08:114	Unconfirmed		
B*08:19N	Unconfirmed	B*08:65	Unconfirmed	B*08:115	Unconfirmed		
B*08:20	Confirmed	B*08:66	Unconfirmed	B*08:116	Unconfirmed		

¹ Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2014-July-25, release 3.17.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-B*08 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

HLA-B*08 SSP subtyping

Specificities and sizes of the PCR products of the 47+1 primer mixes used
for HLA-B*08 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*08 alleles ³	Other amplified HLA Class I alleles ⁴
1	215 bp	800 bp	*08:01:01-08:05, 08:07-08:08N, 08:10-08:11, 08:14-08:15, 08:17-08:19N, 08:21-08:24, 08:26:01-08:39, 08:41-08:48, 08:50-08:54, 08:56:01-08:59:02, 08:61-08:69, 08:71-08:78, 08:80-08:83, 08:85-08:88, 08:90-08:106, 08:108-08:128, 08:130-08:134	*07:04, 07:19, 07:25, 07:146, 35:87, 37:09, 41:02:01-41:02:05, 41:04, 41:10-41:11, 41:13, 41:15, 41:18-41:19, 41:23-41:24, 41:27, 41:31, 42:01:01-42:02, 42:05:01-42:12, 42:14-42:18, 42:20, 44:106, 44:158, 44:166
2	215 bp	800 bp	*08:01:01-08:03, 08:05, 08:07-08:16, 08:18-08:25, 08:27-08:49, 08:51-08:53:02, 08:55-08:61, 08:63-08:64, 08:66-08:84, 08:86N-08:93, 08:95-08:109, 08:111-08:122, 08:124-08:131, 08:133-08:134	*15:180, 35:218, 35:256, 51:68, 51:176
3	245 bp	1070 bp	*08:01:01-08:01:30, 08:04-08:05, 08:07-08:14, 08:16-08:25, 08:27-08:49, 08:51, 08:53:01-08:61, 08:63-08:77, 08:79-08:93, 08:95-08:116, 08:118-08:125, 08:127-08:130, 08:132-08:134	*07:29, 07:186, 14:28, 15:142, 15:180, 35:218, 35:256, 39:12, C*07:124, C*07:155
4	245 bp	1070 bp	*08:02, 08:113, 08:117	*44:49, 44:156
5	245 bp	1070 bp	*08:03, 08:23, 08:52, 08:54, 08:78, 08:110, 08:133	*15:142, 35:218, 35:256, 49:25, 51:68, 51:176, 57:49
6⁶	205 bp	1070 bp	*08:04, 08:17, 08:24, 08:54, 08:110	*15:142, 44:49, 44:156, 49:25
7⁶	215 bp 250 bp	1070 bp	*08:05 *08:03, 08:36, 08:78	*49:25, 51:68, 51:176, 57:49, C*07:377
8⁷	170 bp	1070 bp	*08:20, 08:40, 08:70, 08:79	*07:05:01-07:06, 07:32, 07:40, 07:53, 07:69, 07:78, 07:90, 07:97, 07:105, 07:112, 07:123, 07:137-07:138, 07:140, 07:176, 07:182N, 07:201N, 07:206-07:207, 07:210, 07:213-07:214, 07:222, 13:18,

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9⁵	115 bp	1070 bp	*08:49, 08:71	*13:46, 15:51, 15:83, 15:179:01-15:179:02, 15:199, 15:218Q, 18:56, 27:47, 27:127, 35:38, 35:115, 35:169, 37:01:01-37:01:09, 37:03N-37:06:02, 37:08, 37:10-37:11, 37:13-37:38, 37:41-37:52, 38:30, 41:24, 42:10, 42:17, 44:02:01:01-44:02:33, 44:05:01-44:06, 44:08-44:09, 44:11-44:12, 44:14-44:25, 44:27:01-44:27:02, 44:33-44:34:02, 44:41:01-44:42, 44:44, 44:48-44:49, 44:51-44:52N, 44:55, 44:58N-44:59:02, 44:62-44:63, 44:66-44:68, 44:70-44:73, 44:75, 44:78, 44:80, 44:83-44:84:02, 44:86-44:91, 44:93, 44:95, 44:97, 44:99-44:102, 44:104, 44:106, 44:112-44:113, 44:116-44:119, 44:123, 44:126:01-44:127, 44:131-44:134, 44:136-44:140, 44:142, 44:145, 44:148-44:149N, 44:151-44:152, 44:158, 44:162, 44:168-44:173, 44:176-44:177, 44:179, 44:185, 44:187, 44:190-44:191, 44:195N-44:196, 44:200-44:201, 45:01:01-45:14, 51:08:01-51:08:02, 51:20, 51:36, 51:44N, 51:97, 51:141, 51:153, 52:19, 53:22, 55:56, 56:13, 57:09, 57:24, 82:01-82:03, 83:01
	165 bp		*08:07, 08:37, 08:89, 08:107	*07:04, 07:19, 07:25, 07:146, 15:51, 15:179:01-15:179:02, 15:199, 15:218Q, 18:56, 35:38, 35:115, 35:169, 35:260, 37:09, 37:35, 41:04, 41:18, 41:21, 42:09, 42:16, 44:17, 44:51, 44:123, 45:09, 53:22, 57:24
10⁵	85 bp 170 bp	800 bp	*08:40 *08:08N	*27:83, 41:19
11	290 bp	800 bp	*08:09, 08:27, 08:84	*41:01:01-41:01:02, 41:05-41:07, 41:12, 41:14, 41:16-41:17, 41:20-41:22, 41:25-41:26, 41:28, 41:30, 41:33, 42:04, 55:20
12	385 bp	1070 bp	*08:10	*39:78

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13⁵	85 bp 290 bp	1070 bp	*08:11 *08:49, 08:60, 08:76, 08:129	*42:13, 53:15
14⁵	115 bp 280 bp	1070 bp	*08:43 *08:12:01-08:12:03, 08:16, 08:49, 08:89, 08:107, 08:129	*41:03:01-41:03:02, 42:13, 53:15
15⁵	75 bp	1070 bp	*08:01:01-08:05, 08:07- 08:25, 08:27-08:49, 08:50 ^w , 08:51-08:55, 08:57-08:61, 08:62 ^w , 08:63-08:65, 08:67N- 08:93, 08:96-08:101, 08:103-08:121, 08:123- 08:134	*07:29, 07:86 ^w , 14:11 ^w , 14:28, 15:03:01 ^w -15:03:05 ^w , 15:09 ^w - 15:10:03 ^w , 15:18:01 ^w -15:18:06 ^w , 15:23 ^w , 15:29 ^w , 15:37 ^w , 15:47:01 ^w - 15:47:02 ^w , 15:49 ^w , 15:51 ^w -15:52 ^w , 15:54 ^w , 15:61 ^w -15:62 ^w , 15:64:01 ^w - 15:64:02 ^w , 15:69 ^w , 15:72 ^w , 15:74 ^w , 15:80 ^w , 15:90 ^w -15:91 ^w , 15:93 ^w , 15:98 ^w -15:99 ^w , 15:103 ^w , 15:108 ^w , 15:114 ^w -15:115 ^w , 15:119 ^w , 15:123 ^w - 15:124 ^w , 15:127 ^w , 15:131 ^w -15:134 ^w , 15:151 ^w , 15:153 ^w , 15:156 ^w , 15:158 ^w , 15:161 ^w , 15:173 ^w , 15:176 ^w , 15:180, 15:186 ^w , 15:197 ^w -15:198 ^w , 15:200 ^w , 15:210 ^w , 15:220 ^w -15:221 ^w , 15:226N ^w , 15:229 ^w , 15:235 ^w , 15:238 ^w , 15:242 ^w , 15:243 ^w , 15:251 ^w - 15:253 ^w , 15:255 ^w , 15:263 ^w , 15:266 ^w , 15:274 ^w -15:275 ^w , 15:281 ^w -15:282 ^w , 15:290 ^w , 15:292 ^w -15:294N ^w , 15:306 ^w -15:307 ^w , 15:311 ^w -15:314 ^w , 15:323 ^w , 15:329 ^w , 18:25 ^w , 18:68 ^w , 35:25 ^w , 35:124 ^w -35:125 ^w , 35:142 ^w , 38:33 ^w , 39:04 ^w , 40:12 ^w , 41:22 ^w , 44:156, 48:22 ^w , 49:25, 51:176, 51:179 ^w , 52:16 ^w , 52:27 ^w , 54:21 ^w , 56:16 ^w , 57:58 ^w , 58:12 ^w , 83:01 ^w , C*06:125
16⁵	105 bp	800 bp	*08:13, 08:25, 08:55	*27:83, 42:19, 53:15
17⁵	95 bp	1070 bp	*08:14, 08:87	*07:28, 07:118, 27:32, 40:180, 44:54, 44:106, 44:135
18	425 bp 470 bp	800 bp	*08:15 *08:39	*07:13, 07:15, 07:92, 39:27, 40:73, 67:02, 73:01-73:02 *07:93, 38:27
19	245 bp 415 bp	1070 bp	*08:44 *08:16, 08:123	*07:13, 07:15, 07:160, 42:18, 67:02
20⁷	140 bp 205 bp	1070 bp	*08:22 *08:17, 08:38, 08:54	*15:142, 44:49, 44:156, 49:25
21	465 bp	1070 bp	*08:18	*07:102, 73:01-73:02
22	130 bp 215 bp	800 bp	*08:19N *08:109	
23^{5,8}	85 bp 235 bp	800 bp	*08:21, 08:25 *08:17, 08:47	*41:17, 53:15 *13:39, 13:67, 15:57, 15:135, 35:27, 35:56, 35:185, 35:203, 44:75, 55:12, 56:41, 78:06, C*05:30, C*06:125

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24 ⁵	85 bp 215 bp	1070 bp	*08:101 *08:26:01-08:26:02, 08:50, 08:94	*51:39 *07:08, 07:32, 07:38, 07:207, 15:08:01-15:08:02, 15:29, 15:56, 15:191, 15:236, 35:01:01:01-35:19, 35:21-35:25, 35:27, 35:29:01-35:49, 35:51-35:62, 35:64:01-35:71, 35:74- 35:75, 35:77, 35:79-35:81, 35:83, 35:86-35:94, 35:96-35:134N, 35:136-35:161, 35:163-35:185, 35:187-35:216N, 35:219-35:225, 35:227-35:228, 35:230, 35:232- 35:254, 35:257-35:260, 38:06-38:07, 39:58, 39:83, 44:06, 48:06, 48:31, 51:01:01-51:06:02, 51:08:01-51:21, 51:23-51:24:05, 51:26-51:46, 51:48- 51:67, 51:69-51:102, 51:104-51:115, 51:117-51:127, 51:129-51:144, 51:146-51:150, 51:152-51:166, 51:168-51:175, 51:177-51:179, 52:06:01-52:06:02, 53:01:01- 53:08:02, 53:10-53:16, 53:18-53:27, 53:29-53:37, 59:01:01:01-59:07, 78:01:01-78:02:02, 78:04, 78:07
25 ⁵	115 bp	1070 bp	*08:49, 08:71	*13:46, 15:51, 15:83, 15:179:01- 15:179:02, 15:199, 15:218Q, 18:56, 27:47, 27:127, 35:38, 35:115, 35:169, 37:01:01-37:01:09, 37:03N- 37:06:02, 37:08, 37:10-37:11, 37:13- 37:38, 37:41-37:52, 38:30, 41:24, 42:10, 42:17, 44:02:01:01-44:02:33, 44:05:01-44:06, 44:08-44:09, 44:11- 44:12, 44:14-44:25, 44:27:01- 44:27:02, 44:33-44:34:02, 44:41:01- 44:42, 44:44, 44:48-44:49, 44:51- 44:52N, 44:55, 44:58N-44:59:02, 44:62-44:63, 44:66-44:68, 44:70- 44:73, 44:75, 44:78, 44:80, 44:83- 44:84:02, 44:86-44:91, 44:93, 44:95, 44:97, 44:99-44:102, 44:104, 44:106, 44:112-44:113, 44:116- 44:119, 44:123, 44:126:01-44:127, 44:131-44:134, 44:136-44:140, 44:142, 44:145, 44:148-44:149N, 44:151-44:152, 44:158, 44:162, 44:168-44:173, 44:176-44:177, 44:179, 44:185, 44:187, 44:190- 44:191, 44:195N-44:196, 44:200- 44:201, 45:01:01-45:14, 51:08:01- 51:08:02, 51:20, 51:36, 51:44N, 51:97, 51:141, 51:153, 52:19, 53:22, 55:56, 56:13, 57:09, 57:24, 82:01- 82:03, 83:01 *15:51, 15:179:01-15:179:02, 15:199, 15:218Q, 18:56, 35:115, 35:260, 37:35, 41:24, 41:30, 42:09,
	160 bp		*08:28, 08:35, 08:37, 08:89, 08:107	

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				44:17, 45:09, 51:97, 53:22, 57:24, C*07:68, C*07:140, C*07:151, C*07:302
26	390 bp	1070 bp	*08:29	*07:195, 40:281
27⁵	105 bp 140 bp	1070 bp	*08:30N *08:22	
28⁵	85 bp 210 bp	1070 bp	*08:31 *08:66	
29	150 bp 250 bp	1070 bp	*08:32, 08:133 *08:36	*35:218, 35:256, 51:68, 51:176, C*07:209 C*07:377
30	260 bp	1070 bp	*08:82N, 08:86N	
31	380 bp	1070 bp	*08:34	
32	230 bp	1070 bp	*08:01:01-08:05, 08:08N- 08:13, 08:15-08:27, 08:29-08:30N, 08:32- 08:36, 08:38-08:48, 08:50-08:73, 08:75-08:78, 08:80-08:86N, 08:88, 08:90-08:102, 08:104- 08:106, 08:108-08:110, 08:112, 08:114-08:115, 08:117-08:133	*35:87, 41:01:01-41:03:02, 41:05, 41:07, 41:11-41:14, 41:16-41:17, 41:19-41:20, 41:22-41:28, 41:30- 41:31, 41:33, 42:01:01-42:02, 42:04, 42:06-42:07, 42:10-42:13, 42:15, 42:17-42:19, 44:166, 55:20
33	220 bp 285 bp	800 bp	*08:55 *08:42	*38:19, 39:03, 39:14, 39:24:01- 39:24:02, 39:29, 39:37, 39:76
34	205 bp 305 bp	1070 bp	*08:45, 08:84 *08:59:01-08:59:02	*40:228 [?]
35⁵	120 bp 205 bp 235 bp	1070 bp	*08:51 *08:57 *08:113	
36⁵	115 bp 270 bp	800 bp	*08:20, 08:53:01-08:53:02 *08:33, 08:100	*42:19
37	155 bp 190 bp 270 bp	1070 bp	*08:41 *08:105 *08:48	*35:192, 35:240, 51:141
38	180 bp 265 bp	1070 bp	*08:61, 08:105 *08:58	
39⁸	190 bp	1070 bp	*08:46	
40	265 bp	1070 bp	*08:26:01-08:26:02, 08:56:01-08:56:02	*07:56:01-07:56:02, 07:72, 07:206, 38:28, 39:63, 40:21 [?] , 40:58 [?] , 40:137 [?] , 40:248 [?] , 40:282 [?] , 41:12, 49:03
41	180 bp 215 bp	1070 bp	*08:108 *08:62	*18:07:01-18:07:02, 18:29, 18:79, 27:23, 35:50, 35:84, 35:162, 35:217, 35:231, 37:21, 40:07-40:08, 40:13, 40:68, 40:106, 40:162, 40:220, 40:232, 51:103, 51:116, 51:151

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42	260 bp	1070 bp	*08:63, 08:100	
43⁸	185 bp 215 bp	800 bp	*08:64 *08:70	*07:113 *07:55, 07:100, 15:07:01-15:07:03, 15:45, 15:68, 15:126, 15:207, 15:324, 46:12, 48:19, C*02:60
44⁶	170 bp 215 bp	1070 bp	*08:67N *08:65	*14:28, 39:12
45⁵	120 bp 225 bp	1070 bp	*08:68 *08:49, 08:69	*42:06, 42:20 ^w
46	295 bp	1070 bp	*08:104	
47⁹	240 bp	1070 bp	*08:03, 08:72N, 08:86N	*49:25, 51:68, 51:176, 57:49
48¹⁰	Negative Control			

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*08 SSP typings.

When the primers in a primer mix can give rise to HLA-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits, the alleles listed are specified according to amplicon length.

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.

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 internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

³For several HLA Class I alleles 1st and/or 4th exon(s) and beyond, as well as intron 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. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

⁴Due to the sharing of sequence motifs between HLA-B alleles, non-HLA-B*08 alleles will be amplified by primer mixes 1 to 21, 23 to 26, 29, 32 to 34, 36, 37, 40, 41, 43 to 45 and 47. In addition, a few HLA-C alleles will be amplified by primer mixes 3, 7, 8, 15, 23, 25, 29 and 43.

⁵HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁶Primer mixes 6, 7 and 44 may have a tendency of giving rise to primer oligomer formation.

⁷Primer mix 8 may give a lower yield of HLA-specific PCR product than the other B*08 primer mixes.

⁸Primer mixes 39 and 43 may have tendencies of unspecific amplifications.

⁹Primer mix 47 may give rise to a lower yield of HLA-specific PCR product than the other HLA-B*08 primer mixes in the B*08:03 alleles

¹⁰Primer mix 48 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by the control primer pairs matching the human growth hormone gene. HLA-specific PCR product sizes range from 75 to 200 base pairs and the PCR product generated by the HGH positive control primer pair is 430 base pairs.

‘w’, might be weakly amplified.

‘?’, nucleotide sequence information not available for the primer matching sequence.

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Lot-specific information

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	215	215	245	245	245	205	215	170	115	85	290	385
Length of int. pos. control ¹	800	800	1070	1070	1070	1070	1070	1070	1070	800	800	1070
5'-primer(s) ²	363 5'-AgC 3'	97 5'-TCg 3'	97 5'-TCg 3'	97 5'-TCg 3'	97 5'-TCg 3'	97 5'-TCg 3'	97 5'-TCg 3'	412 5'-ATA 3'	412 5'-ATg 3'	475 5'-Cg 3'	355 5'-TCC 3'	44 5'-ggC 3'
3'-primer(s) ³	538 5'-gTC 3'	272 5'-TgA 3'	299 5'-TCT 3'	309 5'-gTg 3'	292 5'-gTA 3'	251 5'-CCg 3'	269 5'-Agg 3'	539 5'-TCC 3'	538 5'-gTC 3'	603 5'-gTg 3'	603 5'-gTg 3'	259 5'-gTC 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
Length of spec. PCR product	85	115	75	105	95	425	245	140	465	130	85	85
Length of int. pos. control ¹	1070	1070	1070	800	1070	800	1070	1070	1070	800	800	1070
5'-primer(s) ²	355 5'-TCA 3'	363 5'-Agg 3'	106 5'-CCA 3'	539 5'-gCT 3'	363 5'-AgC 3'	44 5'-ggC 3'	44 5'-ggC 3'	97 5'-TCg 3'	44 5'-ggC 3'	636 5'-CAC 3'	106 5'-CCA 3'	97 5'-TCT 3'
3'-primer(s) ³	603 5'-gTg 3'	603 5'-gTg 3'	142 5'-TgA 3'	603 5'-gTg 3'	418 5'-gTC 3'	299 5'-TCA 3'	119 5'-ggg 3'	261 5'-gTC 3'	341 5'-CgT 3'	724 5'-CTA 3'	302 5'-ggT 3'	272 5'-TgA 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
Length of spec. PCR product	115	390	105	85	150	260	380	230	220	205	120	115
Length of int. pos. control ¹	1070	1070	1070	1070	1070	1070	1070	1070	800	1070	1070	800
5'-primer(s) ²	419 5'-gTC 3'	317 5'-gCA 3'	363 5'-AgC 3'	103 5'-CCA 3'	97 5'-TCg 3'	363 5'-AgC 3'	319 5'-gCT 3'	412 5'-ATA 3'	363 5'-AgC 3'	44 5'-ggC 3'	108 5'-ATC 3'	364 5'-gCT 3'
3'-primer(s) ³	538 5'-gTC 3'	419 5'-CgT 3'	426 5'-TCT 3'	272 5'-TgA 3'	206 5'-CCg 3'	573 5'-AgT 3'	412 5'-gTT 3'	603 5'-gTg 3'	544 5'-ggT 3'	85 5'-CAA 3'	272 5'-TgA 3'	603 5'-gTg 3'
Well No.	25	26	27	28	29	30	31	32	33	34	35	36

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Well No.	37	38	39	40	41	42	43	44	45	46	47
Length of spec. PCR product	155	180	190	265	180	260	185	170	120	295	240
	190	265			215		215	215	225		
	270										
Length of int.	1070	1070	1070	1070	1070	1070	800	1070	1070	1070	1070
pos. control ¹											
5'-primer(s) ²	160	139	97	44	97	385	363	97	419	350	97
	5'-ACT 3'	5'-TCg 3'	5'-TCg 3'	5'-ggC 3'	5'-TCC 3'	5'-ggT 3'	5'-AgC 3'	5'-TCg 3'	5'-gTT 3'	5'-TCC 3'	5'-TCg 3'
	376	379			397	388			419		363
	5'-gCA 3'	5'-ACA 3'			5'-gCT 3'	5'-CgA 3'			5'-gTT 3'		5'-AgC 3'
	454	454							523		
	5'-ACC 3'	5'-ACC 3'							5'-CCT 3'		
3'-primer(s) ³	272	272	245	142	272	603	506	225	603	603	292
	5'-TgA 3'	5'-TgA 3'	5'-ACC 3'	5'-TgC 3'	5'-TgA 3'	5'-gTg 3'	5'-Tgg 3'	5'-ATT 3'	5'-gTg 3'	5'-gTg 3'	5'-gTA 3'
	603	603			538		538	272			295
	5'-gTg 3'	5'-gTg 3'			5'-gTC 3'		5'-CCA 3'	5'-TgC 3'			5'-TCA 3'
											573
											5'-AgT 3'
Well No.	37	38	39	40	41	42	43	44	45	46	47

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

²The nucleotide position 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.

³The nucleotide position 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.

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Lot-specific information

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Lot-specific information

CELL LINE VALIDATION SHEET																				
HLA-B*08 SSP subtyping kit ²																				
					Prod. No.:	Well														
						33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
						201318633	201441234	201441235	201441236	201441237	201441238	201318639	201318640	201441241	201318642	201318643	201318644	201318645	201441246	201441247
	IHCW cell line ¹			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	9007	DEM	*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		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Lot-specific information

¹The provided cell line HLA specificities are retrieved from the <http://www.ihwg.org/hla> web site. The specificity of an individual cell line may thus be subject to change.

²The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs

No DNAs carrying the alleles to be amplified by primer solutions 5 to 7, 9, 10, 12 to 14, 16, 17, 19, 20, 25 to 31, 33 to 40, 42 and 44 to 47 were available. The specificities of the primers in primer solutions 5 to 7, 9, 10, 13, 14, 16, 17, 19, 20, 25, 29, 33, 35, 36, 40, 44, 45 and 47 were tested by separately adding one or two additional 5'-primers, respectively one or two additional 3'-primers. The specificity of the 5'-primers in primer solutions 12, 27, 30, 34, 39 and 47 were tested by adding one additional 3'-primer, the 3'-primers were not possible to test. In primer solutions 26, 28, 31, 37, 38, 42 and 46 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 4, 6, 7, 19, 20, 22, 29, 33, 35, 43, 44 and 47 one or two of the 3'-primers were not possible to test, and in primer solutions 10, 13, 14, 35, 36 and 45 one of the 5'-primers was not possible to test. Additional primers in primer solutions 4, 11, 18, 23, 24 and 41 were tested by separately adding one additional 3'-primer respective one additional 5'-primer.

101.513-24/04– including *Taq* pol., IFU-01
101.513-24u/04u – without *Taq* pol., IFU-02

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Lot-specific information

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