

101.515-12– including *Taq* pol., IFU-01
101.515-12u – without *Taq* pol., IFU-02

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

Lot No.: **54Y**

Lot-specific information
Olerup SSP® HLA-B*13

Product number:	101.515-12– including <i>Taq</i> pol. 101.515-12u – without <i>Taq</i> pol.
Lot number:	54Y
Expiry date:	2017-December-01
Number of tests:	12
Number of wells per test:	30+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. 54Y.

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*13 Lot (56V)**

The HLA-B*13 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.

Two wells have been added to HLA-B*13, wells **30 to 31**.

¹As described in section Uniquely Identified Alleles.

The HLA-B*13 primer set, specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP®* HLA-B*13 lot was made (**Lot No. 56V**). The kit design is based on IMGT/HLA database 3.20.0

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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.

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

Well	5'-primer	3'-primer	rationale
6	-	Added	3'-primer added for the B*13:56:02N allele.
8	Moved	Moved	Primer pair moved to well 30 for decreased tendency of primer oligomer formation.
12	Modified	-	5'-primer modified for increased HLA-specific amplification.
13	Modified	Added	5'-primer modified for increased HLA-specific amplification, 3'-primer added for the B*13:57 allele.
15	Moved	Moved	Primer pair for B*13:55 moved to well 29.
16	-	Added	3'-primer added for the B*13:57 allele.
19	Added	-	5'-primer added for the B*13:76N
29	Added	Added	Updated negative control moved to well 31, primer pair added from well 15 for the B*13:55 allele.
30	Added	Added	Primer pair added from well 8.
31	-	-	Updated negative control added from well 29.

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Well **31** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup* SSP® HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all 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.
The PCR product generated by the positive control primer pair is 430 base pairs.

Length of PCR product	105	200	105	80	75	80	85
5'-primer¹	164	340	440	45	45	43	36
	5'-CAC ^{3'}	5'-Agg ^{3'}	5'-TTA ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-TAC ^{3'}
							36
							5'-TAT ^{3'}
3'-primer²	231	2nd I	507	59	58	57	47
	5'-TgC ^{3'}	5'-AAA ^{3'}	5'-TTg ^{3'}	5'-CTC ^{3'}	5'-ggC ^{3'}	5'-CTC ^{3'}	5'-ACA ^{3'}
							48
							5'-gCA ^{3'}
							48
							5'-gCC ^{3'}
							52
							5'-TgT ^{3'}
A*	+	+	+				
B*	+	+	+				
C*	+	+	+				
DRB1				+	+		
DRB3				+	+		
DRB5				+			
DQB1					+		
DPB1						+	
DQA1							+

¹The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and codon 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 for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and codon 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

PRODUCT DESCRIPTION

HLA-B*13 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*13:01 to B*13:83 alleles.

PLATE LAYOUT

Each test consists of 31 PCR reactions in a 32 well PCR plate.

Well 32 is 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	NC	empty

The 32 well cut PCR plate is marked with 'HLA-B*13' in silver/gray ink.

Well No. 1 is marked with the Lot No. '54Y'.

Wells 1 to 30 – HLA-B*13 high resolution primers.

Well 31 – 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 heat-sealed with a PCR-compatible foil.

Please note: When removing each 32 well PCR plate, make sure that the remaining plates stay sealed. 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*13 alleles will be amplified by primer mixes 1 to 16, 20, 23, 24, 26 and 29. In addition, a few HLA-C alleles will be amplified by primer mixes 3, 5, 7, 10, 12, 15 and 19.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*13 alleles, i.e. **B*13:01 to B*13:83**, recognized by the HLA Nomenclature Committee in April 2015^{1,2} will be amplified by the primers in the HLA-B*13 subtyping kit.

The HLA-B*13 kit enables separation of the confirmed HLA-B*13 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*13 alleles is listed below.

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The HLA-B*13 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 HLA-B*13 kit cannot distinguish the silent mutations in the B*13:01:01-13:01:10 alleles, the 13:02:01-13:02:18 alleles, the B*13:13:01-13:13:02 alleles, the B*13:26:01-13:26:02 alleles or the B*13:56:01N-13:56:02N alleles.

The following HLA-B*13 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix
B*13:12:01, 13:52	13
B*13:15, 13:33	16
B*13:27, 13:49N	17
B*13:34, 13:38	19

¹HLA-B alleles listed on the IMGT/HLA web page 2015-April-17, release 3.20.0, 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>.

ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
B*13:01:01	Confirmed	B*13:06	Confirmed	B*13:33	Unconfirmed	B*13:62	Unconfirmed
B*13:01:02	Unconfirmed	B*13:07N	Unconfirmed	B*13:34	Unconfirmed	B*13:63N	Unconfirmed
B*13:01:03	Unconfirmed	B*13:08	Confirmed	B*13:35	Unconfirmed	B*13:64	Confirmed
B*13:01:04	Unconfirmed	B*13:09	Unconfirmed	B*13:36	Unconfirmed	B*13:65	Unconfirmed
B*13:01:05	Unconfirmed	B*13:10	Confirmed	B*13:37	Unconfirmed	B*13:66	Unconfirmed
B*13:01:06	Unconfirmed	B*13:11	Confirmed	B*13:38	Unconfirmed	B*13:67	Unconfirmed
B*13:01:07	Unconfirmed	B*13:12:01	Confirmed	B*13:39	Unconfirmed	B*13:68	Unconfirmed
B*13:01:08	Unconfirmed	B*13:12:02	Unconfirmed	B*13:40	Unconfirmed	B*13:69	Unconfirmed
B*13:01:09	Unconfirmed	B*13:13:01	Confirmed	B*13:41	Unconfirmed	B*13:70	Confirmed
B*13:01:10	Unconfirmed	B*13:13:02	Unconfirmed	B*13:42	Unconfirmed	B*13:71	Unconfirmed
B*13:02:01	Confirmed	B*13:14	Unconfirmed	B*13:43	Unconfirmed	B*13:72	Unconfirmed
B*13:02:02	Confirmed	B*13:15	Unconfirmed	B*13:44	Unconfirmed	B*13:73	Unconfirmed
B*13:02:03	Unconfirmed	B*13:16	Unconfirmed	B*13:45	Confirmed	B*13:74	Unconfirmed
B*13:02:04	Confirmed	B*13:17	Unconfirmed	B*13:46	Unconfirmed	B*13:75	Unconfirmed
B*13:02:05	Unconfirmed	B*13:18	Unconfirmed	B*13:47	Confirmed	B*13:76N	Unconfirmed
B*13:02:06	Unconfirmed	B*13:19	Unconfirmed	B*13:48	Confirmed	B*13:77	Unconfirmed
B*13:02:07	Unconfirmed	B*13:20	Unconfirmed	B*13:49N	Unconfirmed	B*13:78	Unconfirmed
B*13:02:08	Unconfirmed	B*13:21	Unconfirmed	B*13:50	Confirmed	B*13:79	Unconfirmed
B*13:02:09	Unconfirmed	B*13:22:01	Confirmed	B*13:51	Unconfirmed	B*13:80	Unconfirmed
B*13:02:10	Unconfirmed	B*13:22:02	Unconfirmed	B*13:52	Unconfirmed	B*13:81	Unconfirmed
B*13:02:11	Confirmed	B*13:23	Unconfirmed	B*13:53	Unconfirmed	B*13:82	Unconfirmed
B*13:02:12	Unconfirmed	B*13:25	Unconfirmed	B*13:54	Unconfirmed	B*13:83	Unconfirmed
B*13:02:13	Unconfirmed	B*13:26:01	Unconfirmed	B*13:55	Confirmed		
B*13:02:14	Confirmed	B*13:26:02	Unconfirmed	B*13:56:01N	Unconfirmed		
B*13:02:15	Unconfirmed	B*13:27	Unconfirmed	B*13:56:02N	Unconfirmed		
B*13:02:16	Unconfirmed	B*13:28	Unconfirmed	B*13:57	Confirmed		
B*13:02:17	Unconfirmed	B*13:29	Unconfirmed	B*13:58	Unconfirmed		
B*13:02:18	Unconfirmed	B*13:30	Unconfirmed	B*13:59	Unconfirmed		
B*13:03	Confirmed	B*13:31	Unconfirmed	B*13:60	Unconfirmed		
B*13:04	Confirmed	B*13:32	Confirmed	B*13:61	Unconfirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2015-April-17, release 3.20.0, www.ebi.ac.uk/imgt/hla.

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RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-B*13 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

HLA-B*13 SSP subtyping

Specificities and sizes of the PCR products of the 30+1 primer mixes used
for HLA-B*13 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*13 alleles ³	Other amplified HLA Class I alleles ⁴
1⁷	140 bp	800 bp	*13:01:01-13:04, 13:06-13:08, 13:10-13:12:01, 13:13:01-13:23, 13:25-13:38, 13:40-13:57, 13:59-13:66, 13:68-13:83	*44:60
2^{5,6}	95 bp	800 bp	*13:01:01-13:02:18, 13:07N-13:21, 13:23, 13:25-13:44, 13:47, 13:49N-13:52, 13:54-13:61, 13:63N-13:83	*27:98, 40:91, 51:93
3	245 bp	800 bp	*13:01:01-13:01:10, 13:07N, 13:12:01-13:13:02, 13:17, 13:20-13:23, 13:25-13:26:02, 13:28-13:29, 13:36, 13:39, 13:43, 13:50-13:52, 13:57, 13:60-13:61, 13:63N, 13:73, 13:76N-13:80, 13:83	*07:12, 07:14, 07:18:01-07:18:02, 07:137, 07:162-07:163, 07:199, 07:225, 07:227, 27:19, 27:30, 35:15:01-35:15:02, 35:33, 35:88, 35:230, 40:04, 40:30, 40:34, 40:59, 40:64, 40:68, 40:120, 40:129, 40:137, 40:160:01-40:160:02, 44:16, 44:21, 44:64:01-44:64:02, 44:124, 44:150, 48:17, 57:13, 57:25, 58:37, C*02:62, C*03:34, C*03:142, C*03:261, C*03:272, C*15:42, C*15:111
4	245 bp	1070 bp	*13:02:01-13:02:18, 13:08-13:09, 13:14-13:16, 13:18-13:19, 13:27, 13:30-13:35, 13:37-13:38, 13:40-13:42, 13:44-13:45, 13:47, 13:49N, 13:54-13:56:02N, 13:58-13:59, 13:65-13:70, 13:72, 13:74-13:75, 13:81-13:82	*07:78, 07:84, 27:14, 27:81, 27:130, 39:50, 40:06:01:01-40:06:11, 40:44, 40:53, 40:70:01-40:70:02, 40:75, 40:83, 40:86, 40:93, 40:95-40:96, 40:103, 40:109-40:110, 40:127, 40:131, 40:148, 40:159, 40:161-40:162, 40:165, 40:167, 40:177, 40:190, 40:230, 40:244, 40:256N, 40:268-40:270, 40:275, 40:294-40:295, 40:298, 45:10, 49:07, 50:31, 51:10, 51:16, 51:31, 51:34, 51:82, 51:93, 52:08, 54:26, 55:09, 55:22, 55:24, 59:09, 73:01-73:02, 78:08
5^{5,7}	125 bp	800 bp	*13:03, 13:22:01-13:22:02, 13:45, 13:48	*07:78, 15:42, 15:73, 15:86, 15:224, 15:303, 35:60, 40:71, 44:10, 46:11, 46:18, 51:62, 51:106, 51:157, 52:25, 54:01:01-54:03, 54:05N, 54:07-54:08N, 54:10, 54:12-54:13, 54:17-54:34, 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, 55:33-55:40, 55:42-55:45, 55:47-55:48, 55:52-55:57, 55:59-55:67, 55:69-55:71, 55:73, 56:01:01:01-56:01:04, 56:01:06-56:02, 56:04, 56:07-56:08, 56:10, 56:13-56:14, 56:16-56:17, 56:19N-56:20:02, 56:23-56:30, 56:33-56:47, 59:01:01:01-59:01:01:02, 59:04-59:09, 82:01-82:03, C*01:90, C*15:91

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	155 bp		*13:16, 13:20, 13:31, 13:41, 13:48	*07:78, 15:42, 15:73, 15:303, 51:62, 51:106, 52:25, 54:20, 54:33, 55:01:01-55:01:14, 55:03, 55:05, 55:09, 55:11, 55:15, 55:21, 55:24-55:25, 55:29, 55:31, 55:33, 55:36, 55:38, 55:40, 55:44-55:45, 55:52-55:55N, 55:59-55:60, 55:64, 55:66, 55:73, 56:25, 56:37, 56:43, C*01:90, C*15:91
	180 bp		*13:03, 13:48	*15:73, 15:303, 40:71, 44:10, 46:11, 51:62, 51:106, 52:25, 54:03, 54:33, 56:01:01:01- 56:01:04, 56:01:06-56:02, 56:04, 56:07- 56:08, 56:13-56:14, 56:16-56:17, 56:20:01- 56:20:02, 56:24-56:30, 56:33-56:47, 59:04, 82:01-82:03
6^{5,9}	105 bp	1070 bp	*13:04, 13:46, 13:56:01N- 13:56:02N, 13:72	*15:04:01-15:04:02, 15:16:01-15:16:03, 15:67, 15:95, 15:137, 15:155, 15:222, 15:254, 15:293, 15:310, 35:37, 35:235, 35:265, 35:276, 39:06:01-39:06:05, 39:33- 39:34, 39:50, 39:57, 39:62, 39:64, 39:83, 39:90, 39:102, 40:86, 40:159, 41:30, 45:02, 45:09, 46:32, 49:04:01-49:05, 50:09, 50:33, 51:13:01-51:13:02, 51:37, 51:63, 51:92:01- 51:92:02, 51:97, 51:172, 52:14, 52:39, 54:14, 55:13, 55:23, 55:32, 55:58, 55:68, 56:22, 59:03, 73:01-73:02
7⁸	190 bp	1070 bp	*13:06, 13:53	*07:34, 08:01:01-08:01:22, 08:01:24-08:05, 08:08N-08:10, 08:12:01-08:13, 08:15-08:20, 08:22-08:24, 08:26:01-08:27, 08:29-08:36, 08:38-08:39, 08:41-08:48, 08:50-08:73, 08:75-08:78, 08:80-08:86N, 08:88, 08:90- 08:106, 08:108 ^w , 08:109-08:110, 08:112- 08:124, 08:126-08:147, 14:13, 15:42, 15:83, 15:86, 15:188, 15:224, 15:252, 18:26, 18:61, 18:97, 35:60, 35:87, 35:157 ^w , 35:276, 37:12, 38:01:01, 38:01:03-38:02:05, 38:02:07-38:07, 38:09-38:21, 38:23-38:28, 38:31-38:51, 38:53-38:56, 39:01:01:01-39:01:01:03, 39:01:03-39:01:18, 39:01:20-39:06:05, 39:08- 39:14, 39:16, 39:18-39:20, 39:22-39:24:02, 39:26-39:28, 39:30-39:31, 39:34-39:37, 39:39:01-39:39:02, 39:41-39:42, 39:44-39:46, 39:48, 39:51-39:54, 39:56-39:73, 39:75- 39:90, 39:92-39:95N, 39:97N, 39:98 ^w , 39:99- 39:101, 40:39, 40:51, 40:163, 40:184, 40:199, 41:01:01-41:03:02, 41:05-41:09, 41:11-41:16, 41:20, 41:22-41:36, 42:01:01- 42:02, 42:04-42:08, 42:10-42:15, 42:17- 42:19, 44:166, 46:18, 48:37, 51:21, 51:36, 51:101, 51:143, 51:153, 51:157, 52:37, 54:01:01-54:02, 54:04-54:05N, 54:07- 54:08N, 54:10-54:13, 54:17-54:25, 54:27- 54:32, 54:34, 55:01:01-55:05, 55:07, 55:10- 55:13, 55:15-55:21, 55:25-55:26, 55:28- 55:40, 55:42-55:45, 55:47-55:50, 55:52- 55:57, 55:59-55:67, 55:69-55:71, 55:73, 56:10, 56:12, 56:23, 58:20, 59:01:01:01-59:03, 59:05-59:08,

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8⁸	225 bp	1070 bp	*13:18, 13:31, 13:41	67:01:01-67:05, 78:07, C*01:90, C*15:24 *07:78, 07:84, 15:04:01-15:04:02, 15:137, 15:303, 35:265, 39:33, 40:161, 46:32, 51:05, 51:29, 51:54, 51:61:01-51:61:02, 51:82, 52:21, 52:24, 54:10, 54:20, 54:33, 55:09, 55:21, 55:37, 55:52, 56:43
9	130 bp 230 bp	1070 bp	*13:42 *13:08	*51:38
10⁵	105 bp	800 bp	*13:21, 13:71	*07:05:01-07:05:07, 07:06, 07:32, 07:34, 07:40, 07:53, 07:69, 07:78, 07:80, 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, 07:209-07:210, 07:213, 07:222, 07:249, 08:01:02, 08:01:13, 08:20, 08:53:02, 08:79, 15:150, 15:188, 27:07:01- 27:07:04, 27:11, 27:20, 27:24, 27:27, 27:33, 27:43, 27:125, 27:130, 35:09:02-35:09:03, 35:18, 35:31, 35:88, 35:230, 40:01:01- 40:01:13, 40:01:15-40:01:20, 40:01:22- 40:01:25, 40:01:27, 40:01:29, 40:01:31, 40:01:33-40:02:09, 40:02:11-40:02:12, 40:02:14-40:02:17, 40:02:19, 40:02:21- 40:02:23, 40:04-40:06:04, 40:06:06-40:06:09, 40:06:11-40:08, 40:10:01-40:13, 40:14:03- 40:16, 40:19, 40:21-40:23, 40:25, 40:27:01- 40:27:02, 40:29-40:30, 40:34-40:35:01, 40:36-40:40, 40:43, 40:45-40:51, 40:53- 40:58, 40:61-40:70:02, 40:72:02-40:76, 40:79-40:86, 40:87:02-40:88, 40:90-40:94, 40:96-40:97, 40:99-40:114:01, 40:115- 40:119, 40:122-40:123, 40:125-40:130:02, 40:132-40:135, 40:137-40:157, 40:160:01- 40:171, 40:173-40:179, 40:182-40:183, 40:185-40:186, 40:188-40:236, 40:239- 40:266, 40:268-40:279, 40:281-40:283, 40:285-40:299, 40:301-40:304, 41:08, 44:31, 48:01:01-48:01:06, 48:03:01-48:07, 48:09- 48:11, 48:13, 48:15-48:16, 48:18-48:22, 48:24, 48:28-48:37, 51:01:09, 51:01:21, 51:01:25, 51:10, 51:24:02-51:24:04, 51:81, 54:11, 55:04, 55:08, 55:49, 56:20:02, 58:27, 67:03, 81:01-81:07, C*01:90, C*02:68, C*15:24 *07:177, 08:102, 08:122, 15:53, 15:183, 15:212, 15:336, 15:345, 18:80, 18:102, 18:107, 27:08, 27:12, 27:18, 27:26, 27:33, 27:40, 27:42, 27:44, 27:89, 35:63, 35:84, 35:90, 35:150:01-35:150:02, 35:184, 35:201, 35:217, 40:01:01-40:01:10, 40:01:12-40:09, 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- 40:57, 40:59-40:75, 40:77-40:95, 40:97- 40:108, 40:111-40:116, 40:118N-40:136, 40:138-40:148, 40:150-40:156, 40:158- 40:187, 40:189-40:200, 40:202-40:214,
	210 bp		*13:09, 13:39, 13:67	

101.515-12– including *Taq* pol., IFU-01
101.515-12u – without *Taq* pol., IFU-02

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Lot-specific information

				40:216N-40:247, 40:249-40:281, 40:283-40:291N, 40:293-40:299, 40:301-40:304, 41:01:01-41:04, 41:06-41:11, 41:13-41:21, 41:23-41:25, 41:27-41:35, 44:09, 44:46, 44:75, 44:90, 44:129, 44:131, 45:01:01-45:15, 46:10, 47:02, 47:03 ^w , 50:01:01-50:02, 50:04-50:20, 50:31-50:42, 56:36, 73:01-73:02
11	280 bp	800 bp	*13:04, 13:10, 13:26:01-13:26:02, 13:72	*07:09, 07:11, 07:17, 07:162, 07:235, 07:237, 07:255, 08:28, 08:35, 08:37, 08:89, 08:107, 15:01:01:01-15:01:04, 15:01:06-15:08:02, 15:11:01-15:16:03, 15:18:01-15:21, 15:23-15:29, 15:31-15:36, 15:38:01-15:40, 15:43-15:44, 15:46-15:47:02, 15:49-15:57, 15:60-15:62, 15:64:01-15:72, 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-15:215, 15:217-15:223, 15:225-15:228, 15:231-15:232, 15:234-15:242, 15:244-15:247, 15:249-15:251, 15:254-15:268, 15:270-15:272N, 15:274-15:286, 15:288-15:302N, 15:304N-15:311, 15:314-15:322, 15:325-15:328, 15:330-15:333, 15:335-15:337, 15:340-15:341, 15:343-15:347, 15:353-15:355, 18:01:01:01-18:15, 18:17N-18:25, 18:27-18:40, 18:42-18:60, 18:62-18:108, 27:41, 27:107, 35:01:01:01-35:01:45, 35:05:01-35:05:03, 35:07-35:08:08, 35:10-35:11:03, 35:14:01-35:17:02, 35:19-35:21, 35:23-35:30, 35:32:01-35:32:02, 35:35, 35:37, 35:40N-35:43:01, 35:45-35:54, 35:57-35:58, 35:61:01-35:64:02, 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, 35:158-35:159, 35:161, 35:165N-35:166, 35:168, 35:170-35:171, 35:173N-35:178, 35:180, 35:185-35:192, 35:194, 35:196-35:197, 35:200, 35:202-35:203, 35:206-35:210, 35:212, 35:215-35:219, 35:222, 35:224-35:229, 35:232, 35:238-35:241, 35:244-35:245, 35:247-35:250, 35:252-35:255, 35:259-35:260, 35:262-35:265, 35:268-35:269, 35:271-35:272, 35:275, 35:277, 35:280, 35:286, 37:35, 38:52, 39:07, 39:43, 39:91, 40:03, 40:20, 40:38, 40:52, 40:59-40:60, 40:105, 40:158-40:159, 40:222, 40:267, 40:284, 41:24, 41:30, 42:09, 44:17, 44:43:01-44:43:02, 44:144, 45:09, 46:01:01-46:10, 46:12-46:17, 46:19-46:60, 46:62-46:63, 48:02:01-48:02:03, 48:14, 48:23, 48:25, 49:04:01-49:05, 50:33, 51:37, 51:45, 51:63, 51:97, 52:39,

101.515-12– including *Taq* pol., IFU-01
101.515-12u – without *Taq* pol., IFU-02

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Lot-specific information

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12^{5,8}	90 bp	1070 bp	*13:11	*07:182N, 15:18:03, 15:73, 15:224, 39:17, 40:48, 40:71, 51:62, 56:02, 56:04, 56:10, C*02:06, C*02:47
13^{5,6}	100 bp	800 bp	*13:19, 13:52, 13:57	*27:04:01-27:04:03, 27:06, 27:20, 27:24-27:25, 27:66N, 27:68-27:69, 27:100, 27:103, 27:105, 49:34
	180 bp		*13:12:01-13:12:02	
14	130 bp	1070 bp	*13:13:01-13:13:02, 13:21, 13:35, 13:59, 13:71	*08:83, 40:91, 51:93, 51:122
15⁵	105 bp	800 bp	*13:22:01	*07:78, 15:73, 15:303, 40:48, 54:22, 56:01:03, 56:20:02, C*15:91
	245 bp		*13:14, 13:83	*07:149, 18:09 ^w , 27:01 ^w , 37:10 ^w , 38:02:01-38:02:06, 38:03-38:04, 38:08, 38:15, 38:18, 38:23, 38:29, 38:35, 38:43-38:50, 40:96 ^w , 40:157 ^w , 40:201 ^w , 44:103 ^w , 44:130, 44:212 ^w
16^{5,8}	90 bp	800 bp	*13:17, 13:33, 13:57	*35:46, 35:207
	205 bp		*13:15	*14:37, 49:09, 51:62, 51:106, 52:25, 56:34
17	160 bp	1070 bp	*13:49N	
	220 bp		*13:27, 13:43	
18	155 bp	1070 bp	*13:28	
	205 bp		*13:32	
	275 bp		*13:44	
19	195 bp	1070 bp	*13:34	*C*17:08
	240 bp		*13:76N	
	340 bp		*13:38	
20⁵	90 bp	1070 bp	*13:36	*35:46
21⁵	120 bp	1070 bp	*13:29	
	180 bp		*13:30, 13:40	
22⁹	135 bp	800 bp	*13:25	
	175 bp		*13:40	
23	130 bp	1070 bp	*13:42	*51:38
	200 bp		*13:37	
	275 bp		*13:44	
24⁷	170 bp	1070 bp	*13:16, 13:20, 13:31, 13:48, 13:62	*07:02:01-07:24, 07:26-07:47, 07:49N-07:50, 07:52-07:154, 07:156-07:163, 07:165-07:201N, 07:203-07:208, 07:210-07:219, 07:221-07:222, 07:224-07:225, 07:227-07:234, 07:236-07:247, 07:249, 07:251N-07:255, 08:20, 08:53:01-08:53:02, 08:79, 14:01:01-14:40, 14:42, 14:44-14:46, 15:01:01:01-15:01:04, 15:01:06-15:04:02, 15:06-15:19, 15:21, 15:23-15:30,

101.515-12– including *Taq* pol., IFU-01
101.515-12u – without *Taq* pol., IFU-02

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46:44-46:63, 47:10, 48:05, 48:08, 48:15,
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51:11N-51:14, 51:16-51:18, 51:21-51:24:05,
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51:45-51:46, 51:48-51:52, 51:55-51:72,
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56:03, 56:05:01-56:06, 56:21, 56:25, 56:32,
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101.515-12– including *Taq* pol., IFU-01
101.515-12u – without *Taq* pol., IFU-02

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Lot No.: **54Y**

Lot-specific information

78:01:01-78:03, 78:05-78:07			
25	160 bp	800 bp	*13:70
	430 bp		*13:64
26⁵	120 bp	1070 bp	*13:50
	175 bp		*13:47
27	175 bp	1070 bp	*13:61, 13:69
28⁵	120 bp	1070 bp	*13:63N
29	255 bp	1070 bp	*13:23, 13:55
			*18:09, 27:01, 37:10, 40:47, 40:96, 40:157, 40:201, 44:15, 44:55, 44:103, 44:188, 44:212, 49:02
30	185 bp	1070 bp	*13:07N
31¹⁰	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*13 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*13 alleles will be amplified by primer mixes 1 to 16, 20, 23, 24, 26 and 29. In addition, a few HLA-C alleles will be amplified by primer mixes 3, 5, 7, 10, 12, 15 and 19.

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

⁶Primer mixes 2 and 13 may give rise to a weak band of about 500 bp. This band should be disregarded when interpreting HLA-B*13 SSP subtypings.

⁷Primer mixes 1, 5 and 24 may give rise to a lower yield of HLA-specific PCR product than the other B*13 primer mixes.

⁸Primer mixes 7, 8, 12 and 16 may have tendencies of unspecific amplifications.

⁹Primer mixes 6 and 22 have a tendency of giving rise to primer oligomer formation.

¹⁰Primer mix 31 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', may be weakly amplified.

101.515-12– including *Taq* pol., IFU-01
101.515-12u – without *Taq* pol., IFU-02

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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	140	95	245	245	125	105	190	225	130	105	280	90
					155				230	210		
					180							
Length of int. pos. control ¹	800	800	800	1070	800	1070	1070	1070	1070	800	800	1070
5'-primer(s) ²	209	506	355	357	420	357	412	357	357	142	419	369
	5'-ggC 3'	5'-gCT 3'	5'-TCA 3'	5'-Tgg 3'	5'-TTA 3'	5'-Tgg 3'	5'-ATA 3'	5'-Tgg 3'	5'-Tgg 3'	5'-TCA 3'	5'-gTC 3'	5'-TAC 3'
										412		
										5'-ATA 3'		
3'-primer(s) ³	309	559	559	559	506	419	559	539	445	311	3 rd I	420
	5'-gTg 3'	5'-CTC 3'	5'-CTC 3'	5'-CTC 3'	5'-TgC 3'	5'-Cgg 3'	5'-CgT 3'	5'-TCC 3'	5'-ggA 3'	5'-ggT 3'	5'-TAT 3'	5'-gCT 3'
					527	419			548	477		
					5'-CCT 3'	5'-CgA 3'			5'-ggC 3'	5'-gCg 3'		
					538	426						
					5'-CCA 3'	5'-TCT 3'						
					559	426						
					5'-CAg 3'	5'-TCC 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	100	130	105	90	160	155	195	90	120	135	130	170
	180		245	205	220	205	240		180	175	200	
						275	340				275	
Length of int. pos. control ¹	800	1070	800	800	1070	1070	1070	1070	1070	800	1070	1070
5'-primer(s) ²	268	419	103	144	368	209	358	209	160	209	357	527
	5'-Agg 3'	5'-gTA 3'	5'-CCT 3'	5'-gCA 3'	5'-gTT 3'	5'-ggC 3'	5'-TCT 3'	5'-ggC 3'	5'-ACT 3'	5'-ggC 3'	5'-Tgg 3'	5'-TgA 3'
	420		420	420	385	357	406		229	520		
	5'-TTA 3'		5'-TTA 3'	5'-TTA 3'	5'-ggT 3'	5'-Tgg 3'	5'-gCA 3'		5'-TAA 3'	5'-AgA 3'		
	636				441		712		520			
	5'-CAC 3'				5'-TAA 3'		5'-TCT 3'		5'-AgA 3'			
3'-primer(s) ³	317	506	309	193	559	323	559	259	309	302	445	3 rd I
	5'-ggA 3'	5'-TgA 3'	5'-gTg 3'	5'-CgT 3'	5'-CTC 3'	5'-AgC 3'	5'-CTC 3'	5'-gTT 3'	5'-gTg 3'	5'-ggC 3'	5'-ggA 3'	5'-TAT 3'
	476		486	476		523	916		3 rd I	3 rd I	515	
	5'-CCA 3'		5'-gCC 3'	5'-CCA 3'		5'-ACC 3'	5'-gAC 3'		5'-TAT 3'	5'-TAT 3'	5'-CCC 3'	
	559			583		593					593	
	5'-CCC 3'			5'-gTg 3'		5'-CgC 3'					5'-CgC 3'	
	700											
	5'-AgA 3'											
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

101.515-12– including *Taq* pol., IFU-01
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Lot-specific information

Well No.	25	26	27	28	29	30
Length of spec. PCR product	160	120	175	120	255	185
	430	175				
Length of int. pos. control ¹	800	1070	1070	1070	1070	1070
5'-primer(s) ²	209	427	628	506	97	106
	5'-ggC 3'	5'-ACT 3'	5'-CAC 3'	5'-gCT 3'	5'-TCC 3'	5'-CCA 3'
		482	649			
		5'-ggT 3'	5'-ACT 3'			
3'-primer(s) ³	329	559	774	583	309	251
	5'-ggC 3'	5'-CTC 3'	5'-ggT 3'	5'-gTA 3'	5'-gTg 3'	5'-CCC 3'
	353					
	5'-AAA 3'					
Well No.	25	26	27	28	29	30

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.

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

Lot-specific information

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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.

101.515-12– including *Taq* pol., IFU-01
101.515-12u – without *Taq* pol., IFU-02

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Lot-specific information

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 9, 13, 14, 16 to 23, 25 to 28 and 30 were available. The specificities of the primers in primer mixes 13, 14, 16, 20 and 22 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 9, 18, 23, 25, 28 and 30 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 17, 19, 21, 26 and 27 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 13 and 22, one 5'-primer was not possible to test and in primer solutions 6, 13, 16 and 22 one or two 3'-primers were not possible to test.

Additional primers in primer solutions 5 and 15 were tested by separately adding one 5'-primer and/or one 3'-primer.

101.515-12– including *Taq* pol., IFU-01
101.515-12u – without *Taq* pol., IFU-02

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Lot No.: 54Y

Lot-specific information

101.515-12– including *Taq* pol., IFU-01
101.515-12u – without *Taq* pol., IFU-02

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Lot No.: **54Y**

Lot-specific information

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