101.562-06 – including *Taq* polymerase, IFU-01
101.562-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for **"Instructions for Use" (IFU)**

Lot No.: 87V

Lot-specific information

Olerup SSP® HLA-B*52

Product number:	**101.562-06 – including *Taq* polymerase** **101.562-06u – without *Taq* polymerase**
Lot number:	**87V**
Expiry date:	**2017-January- 01**
Number of tests:	**6**
Number of wells per test:	**15+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. 87V.

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*52 LOT (90R).

The HLA-B*52 kit is updated for new alleles to enable separation of:

- Confirmed[1] 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.

[1]As described in section Uniquely Identified Alleles.

The HLA-B*52 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup* SSP® HLA-B*52 lot was made **(Lot No. 90R)**.

Lot No.: 87V **Lot-specific information**

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
4	Added	Added	Primer pair added for the B*52:36 allele, exchanged positive control primer pair.
5	Added	-	5'-primer added from well 7.
6	Added	Added	Primer pair added from well 7.
7	Added, moved	Added, moved	Primer pairs added from well 16, primers moved to wells 5 and 6.
8	-	Added	3'-primer added for the B*52:25 allele.
12	-	Added	3'-primer added for the B*52:01:19 allele.
16	Moved	Moved	Primer pair moved to well 7, Negative Control.

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Lot No.: 87V **Lot-specific information**

Well **16** 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'-primer[1]	**164**	**340**	**440**	**45**	**45**	**43**	**36**
	5'-CAC3'	**5'-Agg3'**	**5'-TTA3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-TAC3'**
							36
							5'-TAT3'
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**	**47**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**	**5'-ACA3'**
							48
							5'-gCA3'
							48
							5'-gCC3'
							52
							5'-TgT3'
A*	+	+	+				
B*	+	+	+				
C*	+	+	+				
DRB1				+	+		
DRB3				+	+		
DRB5				+			
DQB1					+		
DPB1						+	
DQA1							+

[1]The 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 codonnumbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

[2]The 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 No.: 87V **Lot-specific information**

PRODUCT DESCRIPTION

HLA-B*52 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*52:01 to B*52:39 alleles.

PLATE LAYOUT

Each HLA-B*52 test consists of 16 PCR reactions in a 16 well cut PCR plate.

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
9	**10**	**11**	**12**	**13**	**14**	**15**	**16**

The 16 well PCR plate is marked with 'HLA-B*52' in silver/gray ink.
Well No. 1 is marked with the Lot No. '87V'.
Wells 1 to 15 – HLA-B*52 high resolution primers.
Well 16 – 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 16 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 many non-HLA-B*52 alleles will be amplified by all the primer mixes.
In addition, a few HLA-C alleles will be amplified by primer mixes 1 and 5.
For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*52, i.e. **B*52:01 to B*52:39**, recognized by the HLA Nomenclature Committee in April 2014[1,2] will be amplified by the primers in the HLA-B*14 subtyping kit[3].

The HLA-B*52 kit enables separation of the confirmed HLA-B*52 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*52 alleles is listed below.

The HLA-B*52 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*52 primer set cannot distinguish the silent mutations in the B*52:01:01:01-52:01:20 alleles, the B*52:02:01-52:02:02 alleles or the B *52:10:01-52:10:03 alleles.

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Lot No.: 87V **Lot-specific information**

The following HLA-B*52 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix
B*52:04, 52:09	2
B*52:12, 52:27	15
B*52:20, 52:25	8

[1]HLA-B alleles listed on the IMGT/HLA web page 2014-April-14, release 3.16.0, www.ebi.ac.uk/imgt/hla.
[2]Alleles 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.
[3]The HLA-B*52 primer set cannot separate the B*52:01:01:01-52:01:20, 52:22-52:24, 52:30-52:31:02 and 52:33-52:35, 52:37-52:39 alleles and the B*53:28 allele. These alleles can be distinguished by the HLA-B low resolution kit and/or the HLA-B*53 high resolution kit.
The HLA-B*52 primer set cannot separate the B*52:28-52:29 alleles and the B*53:17:01-53:17:02 alleles. These alleles can be distinguished by the HLA-B low resolution kit and/or the HLA-B*53 high resolution kit.

ALLELE CONFIRMATION STATUS

Allele	Status[1]	Allele	Status[1]	Allele	Status[1]	Allele	Status[1]
B*52:01:01:01	**Confirmed**	B*52:01:19	Unconfirmed	B*52:16	Unconfirmed	B*52:35	Unconfirmed
B*52:01:01:02	Unconfirmed	B*52:01:20	Unconfirmed	B*52:17	Unconfirmed	B*52:36	Unconfirmed
B*52:01:01:03	Unconfirmed	**B*52:02:01**	**Confirmed**	B*52:18	Unconfirmed	B*52:37	Unconfirmed
B*52:01:02	**Confirmed**	B*52:02:02	Unconfirmed	B*52:19	Unconfirmed	B*52:38	Unconfirmed
B*52:01:03	**Confirmed**	B*52:03	Unconfirmed	B*52:20	Unconfirmed	B*52:39	Unconfirmed
B*52:01:04	Unconfirmed	B*52:04	Unconfirmed	**B*52:21**	**Confirmed**		
B*52:01:05	**Confirmed**	**B*52:05**	**Confirmed**	B*52:22	Unconfirmed		
B*52:01:06	**Confirmed**	B*52:06:01	Unconfirmed	B*52:23	Unconfirmed		
B*52:01:07	**Confirmed**	**B*52:06:02**	**Confirmed**	B*52:24	Unconfirmed		
B*52:01:08	Unconfirmed	B*52:07	Unconfirmed	**B*52:25**	**Confirmed**		
B*52:01:09	Unconfirmed	**B*52:08**	**Confirmed**	B*52:26	Unconfirmed		
B*52:01:10	Unconfirmed	B*52:09	Unconfirmed	B*52:27	Unconfirmed		
B*52:01:11	Unconfirmed	B*52:10:01	Unconfirmed	B*52:28	Unconfirmed		
B*52:01:12	**Confirmed**	B*52:10:02	Unconfirmed	B*52:29	Unconfirmed		
B*52:01:13	Unconfirmed	B*52:10:03	Unconfirmed	B*52:30	Unconfirmed		
B*52:01:14	**Confirmed**	**B*52:11**	**Confirmed**	B*52:31:01	Unconfirmed		
B*52:01:15	Unconfirmed	**B*52:12**	**Confirmed**	B*52:31:02	Unconfirmed		
B*52:01:16	Unconfirmed	B*52:13	Unconfirmed	B*52:32	Unconfirmed		
B*52:01:17	Unconfirmed	B*52:14	Unconfirmed	B*52:33	Unconfirmed		
B*52:01:18	Unconfirmed	B*52:15	Unconfirmed	B*52:34	Unconfirmed		

[1]Allele status "confirmed" or "unconfirmed" as listed on the IMGT/HLA web page 2014-April-14, release 3.16.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-B*52 homo- and heterozygotes is available upon request.

101.562-06 – including *Taq* polymerase, IFU-01
101.562-06u – without *Taq* polymerase, IFU-02

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Lot No.: 87V **Lot-specific information**

SPECIFICITY TABLE

HLA-B*52 SSP subtyping

Specificities and sizes of the PCR products of the 15+1 primer mixes used for HLA-B*52 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified HLA-B*52 alleles	Other amplified HLA Class I alleles[3,4]
1[5]	95 bp	**800 bp**	*52:01:01:01-52:02:02, 52:04-52:09, 52:12-52:18, 52:20-52:27, 52:30-52:39	*07:218, 13:62, 14:01:01-14:40, 15:37-15:38:02, 18:15, 18:19, 18:30, 18:57, 35:21, 35:96, 35:188, 40:28, 40:51, 40:174, 49:09, 51:01:01-51:01:49, 51:03-51:04, 51:06:01-51:07:02, 51:11N-51:14, 51:16-51:18, 51:21-51:22, 51:24:01-51:24:05, 51:26-51:30, 51:32-51:33, 51:35, 51:37-51:39, 51:41N, 51:43, 51:45-51:46, 51:48-51:52, 51:55-51:58, 51:60-51:72, 51:74-51:80, 51:82-51:92, 51:94-51:96, 51:98N-51:107, 51:109-51:114, 51:116-51:119, 51:121, 51:123-51:138, 51:140, 51:142, 51:145, 51:147, 51:149N-51:152, 51:154-51:156, 51:158-51:173Q, 53:06, 53:28, 56:05:01-56:06, 56:21, 58:08:01-58:08:02, 78:01:01-78:03, 78:05-78:07, **C*01:30, C*08:51, C*12:87, C*15:39**
2	135 bp 235 bp	1070 bp	*52:04 *52:09	 *08:52, 15:87, 53:05, 53:16, 53:33, 57:60
3[5,6]	95 bp	1070 bp	*52:01:01:01-52:01:20, 52:03-52:15, 52:17, 52:19-52:31:02, 52:33-52:39	*13:58, 15:202, 15:239, 18:12, 35:10, 35:13, 35:16, 35:28, 35:69, 35:80, 35:226, 37:01:01-37:01:05, 37:01:07-37:05, 37:07, 37:09-37:27, 37:29-37:49, 40:185, 40:209, 44:83, 44:134, 49:03, 53:17:01-53:17:02, 53:28, 78:05-78:06
4	140 bp 280 bp	**800 bp**	*52:02:01-52:02:02	*15:13:01-15:13:02, 15:16:01-15:17:02, 15:24:01-15:24:02, 15:67, 15:87, 15:95, 15:157, 15:162, 15:168, 15:177, 15:196, 15:208, 15:216, 15:222, 15:230, 15:254, 15:268, 15:273, 51:104, 57:01:01-57:11, 57:13-57:15, 57:17-57:44, 57:46-57:50, 57:52-57:68, 57:70, 58:36 *52:36
5	145 bp 190 bp 225 bp	1070 bp	*52:21 *52:03, 52:11 *52:05	*15:01:02, 15:277-15:278, 51:61:01-51:61:02, **C*02:02:23** *07:02:11, 07:05:03, 15:01:02, 15:09, 15:243, 15:252, 15:277-15:278, 27:04:03, 35:01:10, 35:04:02, 35:251, 40:01:06, 40:26, 40:95, 44:62, 49:18, 50:14, 51:02:01-51:02:03, 51:02:05, 51:05, 51:23, 51:34, 51:36, 51:40, 51:54, 51:59, 51:93, 51:108, 51:143-51:144, 51:146, 55:01:04, 78:04, **C*02:02:23**
6	135 bp	1070 bp	*52:03, 52:05, 52:10:01-52:10:03	*35:01:10, 35:04:02, 35:251, 40:01:06, 51:05[W], 51:09:01-51:09:02, 51:19, 51:31, 51:40, 51:54[W], 51:73, 51:93, 51:122, 51:139, 78:04

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	180 bp 225 bp		*52:15 *52:05	*44:62[W], 51:23[W], 51:108[W]
7[7,8]	160 bp 215 bp	1070 bp	*52:13 *52:19	*08:09, 08:84, 13:46, 15:83, 42:04, 44:20, 51:08:01-51:08:02, 51:20, 51:36, 51:44N, 51:97, 51:141, 51:153, 55:20, 55:56, 56:13
8	145 bp 500 bp	1070 bp	*52:20 *52:25	*13:58, 18:09, 37:10, 44:83, 44:134, 51:54, 51:78:01-51:78:02, 53:09, 53:11-53:13, 53:31 *13:58, 35:60, 49:03, 51:15, 51:62, 51:157
9[5]	100 bp	**800 bp**	*52:06:01, 52:14	*13:46, 15:137, 35:01:08, 35:01:15, 35:29:02, 35:235, 39:06:01-39:06:04, 39:33-39:34, 39:50, 39:57, 39:62, 39:64, 39:83, 39:90, 40:86, 45:02, 50:09, 51:01:33, 51:13:01-51:13:02, 51:92, 51:172, 55:13, 55:58, 56:22, 59:03, 73:01-73:02
10[5]	95 bp 245 bp	1070 bp	*52:18 *52:08	*07:78, 07:84, 13:02:01-13:02:16, 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, 13:54-13:56N, 13:58-13:59, 13:65-13:70, 13:72, 13:74-13:75, 27:14, 27:81, 39:50, 40:06:01:01-40:06:10, 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, 45:10, 49:07, 50:31, 51:10, 51:16, 51:31, 51:34, 51:82, 51:93, 54:26, 55:09, 55:22, 55:24, 73:01-73:02
11[5,7]	85 bp 225 bp	1070 bp	*52:06:01-52:06:02 *52:17	*07:38, 08:02-08:03, 08:52, 08:78, 08:117, 27:23, 37:21, 38:06-38:07, 40:13, 44:06, 51:01:01-51:01:16, 51:01:18-51:06:02, 51:08:01-51:21, 51:23-51:24:05, 51:26-51:46, 51:48-51:117, 51:119-51:173Q, 53:01:01-53:08:02, 53:10-53:16, 53:18-53:27, 53:29-53:35, 59:01:01:01-59:07
12[5]	85 bp	1070 bp	*52:01:01:01-52:05, 52:07-52:17, 52:19-52:39	*13:01:01-13:04, 13:06, 13:08, 13:10-13:18, 13:20-13:23, 13:25-13:35, 13:37-13:38, 13:40-13:66, 13:68-13:78, 15:24:01-15:24:02, 15:36, 15:43, 15:87, 15:157, 15:256, 27:29, 37:01:01-37:01:08, 37:02-37:04:02, 37:06:01-37:07, 37:09-37:10, 37:12-37:20, 37:22-37:36, 37:38-37:49, 38:03, 40:19, 40:47, 40:96, 40:109-40:110, 40:117, 40:157, 40:188, 40:201, 44:02:01:01-44:02:21, 44:02:23-44:05:04, 44:07-44:08, 44:10-44:11, 44:14-44:45, 44:47-44:66, 44:68, 44:70-44:74, 44:76-44:89, 44:91-44:96, 44:98-44:112, 44:114-44:126:02, 44:128, 44:130, 44:132-44:170, 44:172-44:193, 47:01:01:01-47:01:02, 47:03-47:09, 48:18, 49:01:01-49:20, 49:22-49:25, 49:27-49:31, 53:17:01-53:17:02, 53:28

101.562-06 – including *Taq* polymerase, IFU-01
101.562-06u – without *Taq* polymerase, IFU-02

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Lot No.: 87V **Lot-specific information**

13	165 bp	**800 bp**	*52:01:01:01-52:06:02, 52:08-52:39	*15:20, 15:228, 15:305, 35:01:01:01-35:01:27, 35:01:29-35:30, 35:32:01-35:34, 35:36-35:42:02, 35:44-35:45, 35:47-35:56, 35:58-35:59, 35:61:01-35:72, 35:74-35:78, 35:80-35:86, 35:88-35:184, 35:186-35:254, 48:02:01-48:02:03, 51:01:01-51:24:05, 51:26-51:41N, 51:43-51:46, 51:48-51:156, 51:158-51:163, 51:165-51:173Q, 53:01:01-53:35, 56:05:01-56:05:02, 56:21, 56:36, 58:01:01-58:02, 58:04-58:10N, 58:12-58:19, 58:21-58:29, 58:31N-58:59, 67:05, 78:01:01-78:07, 81:03, 83:01?
14[7]	165 bp	1070 bp	*52:07	*07:02:01-07:57, 07:59-07:219, 08:01:01-08:05, 08:07-08:123, 13:01:01-13:04, 13:06-13:23, 13:25-13:60, 13:62-13:78, 14:01:01-14:40, 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, 15:131-15:227, 15:229-15:283, 15:285-15:304N, 15:306-15:320, 18:01:01:01-18:15, 18:17N-18:103, 27:01-27:21, 27:23-27:125, 35:31, 35:35, 35:43:01, 35:46, 35:57, 35:60, 35:79, 35:87, 35:185, 37:01:01-37:49, 38:01:01-38:53, 39:01:01:01-39:01:01:03, 39:01:03-39:20, 39:22-39:45, 39:47-39:92, 40:01:01-40:06:07, 40:06:09-40:16, 40:18-40:40, 40:42-40:235, 40:237-40:271, 40:273-40:285, 41:01-41:32, 42:01:01-42:02, 42:04-42:20, 44:02:01:01-44:65, 44:67-44:117, 44:119-44:193, 45:01:01-45:14, 46:01:01-46:01:16, 46:01:18-46:57, 47:01:01:01-47:09, 48:01:01-48:01:06, 48:02:02-48:34, 49:01:01-49:31, 50:01:01-50:02, 50:04-50:20, 50:31-50:38, 51:42, 51:157, 54:01:01-54:32, 55:01:01-55:05, 55:07-55:64, 56:01:01-56:04, 56:06-56:20:02, 56:22-56:43, 57:01:01-57:01:10, 57:01:12-57:70, 58:11, 58:20, 59:01:01:01-59:08, 67:01:01-67:04, 81:01-81:02, 81:04N-81:07, 82:01-82:03, 83:01?
15[5,6,9]	95 bp	**800 bp**	*52:12, 52:26	*07:84, 13:04, 13:35, 13:72, 15:04:01-15:04:02, 15:16:01-15:16:03, 15:67, 15:95, 15:155, 15:222, 15:254, 15:293, 15:310, 35:37, 35:235, 40:44, 40:159, 41:21, 44:20, 44:47, 44:100, 45:09, 46:32, 49:04-49:05, 50:33, 51:37, 51:90, 51:92, 54:14-54:15, 55:23, 55:27
	170 bp		*52:16, 52:27	*07:27, 07:50, 08:04, 08:17, 08:54, 08:110, 15:03:01-15:03:04, 15:47:01-15:47:02, 15:54, 15:61-15:62, 15:64:01-15:64:02, 15:68-15:69, 15:91, 15:98, 15:103, 15:123, 15:127, 15:131-15:132, 15:151, 15:156, 15:158, 15:173, 15:210, 15:220, 15:235, 15:242, 15:251, 15:253, 15:266, 15:274, 15:281-15:282, 18:01:01:01-18:03, 18:05-18:06, 18:08-18:15, 18:17N-18:28, 18:30-18:32, 18:34-18:78, 18:81-18:97, 18:99-18:101, 18:103,

			37:01:01-37:20, 37:23-37:25, 37:27-37:49, 38:03, 39:02:01-39:02:02, 39:08, 39:13:01-39:13:02, 39:23, 39:39:01-39:39:02, 39:49, 39:88, 40:12, 40:149, 41:22, 42:11, 44:130, 44:156, 48:01:01-48:02:01, 48:02:03-48:05, 48:07-48:30, 48:32-48:34, 49:25
16[10]	-	-	**Negative Control**

[1]Alleles 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*52 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 inherit 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.
[2]The 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.
[3]For 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.
[4]Due to the sharing of sequence motifs between HLA-B alleles many non-HLA-B*52 alleles will be amplified by all the primer mixes.
In addition, a few HLA-C alleles will be amplified by primer mixes 1 and 5.
[5]HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.
[6]Primer mixes 3 and 15 may give rise to a lower yield of HLA-specific PCR product than the other B*52 primer mixes.
[7]Primer mixes 7, 11 and 14 may have tendencies of unspecific amplifications.
[8]Primer mix 7 has a tendency to giving rise to primer oligomer formation.
[9]Primer mix 15 may give rise to a weak band of about 550 bp. This band should be disregarded when interpreting HLA-B*52 SSP subtypings.
[10]Primer mix 16 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by control primer pairs. PCR product sizes range from 75 to 200 base pairs. The PCR product generated by the control primer pair is 430 base pairs.
'?', nucleotide sequence information not available for the primer matching sequence.
'w', might be weakly amplified.

101.562-06 – including *Taq* polymerase, IFU-01
101.562-06u – without *Taq* polymerase, IFU-02

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	95	135	95	140	145	135	160	145	100	95	85	85
PCR product		235		280	190	180	215	500		245	225	
					225	225						
Length of int.	800	1070	1070	800	1070	1070	1070	1070	800	1070	1070	1070
pos. control[1]												
5'-primer(s)[2]	527	106	206	209	134	134	86	206	213	206	133	272
	5' -TgA 3'	5' -CCA 3'	5' -gAC 3'	5' -ggC 3'	5' -CCC 3'	5' -CCC 3'	5' -CAA 3'	5' -gAC 3'	5' -CCg 3'	5' -gAC 3'	5' -CCA 3'	5' -CTC 3'
				652	435	435	362		357	357	272	
				5' -CCg 3'	5' -AAA 3'	5' -AAA 3'	5' -gAC 3'		5' -Tgg 3'	5' -Tgg 3'	5' -CTT 3'	
3'-primer(s)[3]	583	200	259	309	317	317	206	309	272	262	317	317
	5' -gTg 3'	5' -TCT 3'	5' -CTC 3'	5' -ATC 3'	5' -ggA 3'	5' -ggA 3'	5' -CCg 3'	5' -gTg 3'	5' -TgA 3'	5' -TgC 3'	5' -ggA 3'	5' -ggA 3'
		302		891	538	527	538	420	419	559		317
		5' -ggC 3'		5' -CAg 3'	5' -CCA 3'	5' -CCA 3'	5' -gTC 3'	5' -gCT 3'	5' -CgA 3'	5' -CTC 3'		5' -ggA 3'
					583	527						
					5' -gTA 3'	5' -CCA 3'						
						572						
						5' -gCg 3'						
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15
Length of spec.	165	165	95
PCR product			170
Length of int.	800	1070	800
pos. control[1]			
5'-primer(s)[2]	652	652	142
	5' -CCg 3'	5' -CCA 3'	5' -TCT 3'
			357
			5' -Tgg 3'
3'-primer(s)[3]	774	774	272
	5' -ggT 3'	5' -ggT 3'	5' -Tgg 3'
			412
			5' -gTC 3'
Well No.	13	14	15

[1]The 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.

[2]The 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.

[3]The 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.

101.562-06 – including *Taq* polymerase, IFU-01
101.562-06u – without *Taq* polymerase, IFU-02

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CELL LINE VALIDATION SHEET

HLA-B*52 SSP primer set[2]

					Well														
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
				Prod. No.:	201078001	201078002	201078003	201436704	201436705	201436706	201078016	201436708	201078009	201078010	201078011	201436712	201078013	201078014	201078015
	IHWC 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	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	9025	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[1]	*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[1]	*51:01		+	-	-	-	-	-	-	-	-	-	+	-	+	-	-
48	9303	TER-ND	*35:01	*44:03	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-

[1]The 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.

101.562-06 – including *Taq* polymerase, IFU-01
101.562-06u – without *Taq* polymerase, IFU-02

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[2]The 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 2 and 6 to 8 were available. The specificities of the primers in primer solutions 2 and 6 to 8 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 5, 6, 7, 9 and 11 one or two 5'-primers were not possible to test, and in primer solutions 2, 10 and 12 one 3'-primer was not possible to test. Additional primers in primer solutions 4, 5, 9, 10 and 15 were tested by separately adding one additional 5'-primer or 3'-primer.

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ADDRESSES:

Manufacturer:
Olerup SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.
Tel: +46-8-717 88 27
Fax: +46-8-717 88 18
E-mail: info-ssp@olerup.com
Web page: http://www.olerup-ssp.com

Distributed by:
Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.
Tel: +43-1-710 15 00
Fax: +43-1-710 15 00 10
E-mail: support-at@olerup.com
Web page: http://www.olerup.com

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382
Tel: 1-877-OLERUP1
Fax: 610-344-7989
E-mail: info.us@olerup.com
Web page: http://www.olerup.com

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.