

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.: **4D5**

Lot-specific information

Olerup SSP® HLA-B*52

Product number:	101.562-06 – including <i>Taq</i> polymerase 101.562-06u – without <i>Taq</i> polymerase
Lot number:	4D5
Expiry date:	2018-08-01
Number of tests:	6
Number of wells per test:	20+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. 4D5.

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 (87V).

The HLA-B*52 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.

Five wells have been added to HLA-B*52, wells **17 to 21**.

¹As described in section Uniquely Identified Alleles.

The HLA-B*52 primer set, 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. 87V). The kit design is based on IMGT/HLA database 3.22.0.

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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
9	-	Added	3'-primer added for the B*52:49N allele.
15	Moved	Moved	Primer pair moved to well 20 for improved HLA-specific amplification.
16	Added	Added	Updated negative control moved to well 21, primer pair added for improved resolution of the B*52 versus B*53 alleles.
17	New	New	New primer pair added for the B*52:43 allele.
18	New	New	New primer pair added for the B*52:30 allele.
19	New	New	New primer pair added for the B*52:53 allele.
20	Added	Added	Primer pair added from well 15, exchanged of positive control primer pair.
21	-	-	Updated negative control added from well 16.

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Well **21** 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*52 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*52:01 to B*52:56 alleles.

PLATE LAYOUT

Each HLA-B*52 test consists of 21 PCR reactions in a 24 well cut PCR plate. Wells 22 to 24 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	NC	empty	empty	empty

The 24 well PCR plate is marked with 'HLA-B*52' in silver/gray ink.

Well No. 1 is marked with the Lot No. '4D5'.

Wells 1 to 20 – HLA-B*52 high resolution primers.

Well 21 – 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 24 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 primer mixes 1 to 18 and 20.

In addition, a few HLA-C alleles will be amplified by primer mixes 1, 5 and 12.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*52, i.e. **B*52:01 to B*52:56**, recognized by the HLA Nomenclature Committee in October 2015^{1,2} will be amplified by the primers in the HLA-B*52 subtyping kit³.

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.

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The HLA-B*52 primer set cannot distinguish the silent mutations in the B*52:01:01:01-52:01:28 alleles, the B*52:02:01-52:02:02 alleles, the B*52:06:02-52:06:03 alleles, the B*52:10:01-52:10:03 alleles or the B*52:31:01-52:31:02 alleles.

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*52:14, 52:49	9
B*52:20, 52:25	8
B*52:31:01-52:31:02, 53:28	16

¹HLA-B alleles listed on the IMGT/HLA web page 2015-October-10, release 3.22.0, 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*52 primer set cannot separate the B*52:28-52:29 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*52:02:01	Confirmed	B*52:27	Unconfirmed	B*52:56	Unconfirmed
B*52:01:01:02	Confirmed	B*52:02:02	Unconfirmed	B*52:28	Unconfirmed		
B*52:01:01:03	Unconfirmed	B*52:03	Unconfirmed	B*52:29	Unconfirmed		
B*52:01:02	Confirmed	B*52:04	Unconfirmed	B*52:30	Confirmed		
B*52:01:03	Confirmed	B*52:05	Confirmed	B*52:31:01	Confirmed		
B*52:01:04	Unconfirmed	B*52:06:01	Unconfirmed	B*52:31:02	Unconfirmed		
B*52:01:05	Confirmed	B*52:06:02	Confirmed	B*52:32	Unconfirmed		
B*52:01:06	Confirmed	B*52:06:03	Confirmed	B*52:33	Unconfirmed		
B*52:01:07	Confirmed	B*52:07	Unconfirmed	B*52:34	Unconfirmed		
B*52:01:08	Unconfirmed	B*52:08	Confirmed	B*52:35	Unconfirmed		
B*52:01:09	Unconfirmed	B*52:09	Unconfirmed	B*52:36	Unconfirmed		
B*52:01:10	Unconfirmed	B*52:10:01	Unconfirmed	B*52:37	Unconfirmed		
B*52:01:11	Unconfirmed	B*52:10:02	Unconfirmed	B*52:38	Unconfirmed		
B*52:01:12	Confirmed	B*52:10:03	Unconfirmed	B*52:39	Unconfirmed		
B*52:01:13	Confirmed	B*52:11	Confirmed	B*52:40	Unconfirmed		
B*52:01:14	Confirmed	B*52:12	Confirmed	B*52:41	Unconfirmed		
B*52:01:15	Unconfirmed	B*52:13	Unconfirmed	B*52:42	Unconfirmed		
B*52:01:16	Unconfirmed	B*52:14	Unconfirmed	B*52:43	Confirmed		
B*52:01:17	Unconfirmed	B*52:15	Unconfirmed	B*52:44	Unconfirmed		
B*52:01:18	Unconfirmed	B*52:16	Unconfirmed	B*52:45	Unconfirmed		
B*52:01:19	Unconfirmed	B*52:17	Unconfirmed	B*52:46	Unconfirmed		
B*52:01:20	Unconfirmed	B*52:18	Unconfirmed	B*52:47	Unconfirmed		
B*52:01:21	Unconfirmed	B*52:19	Unconfirmed	B*52:48	Unconfirmed		
B*52:01:22	Unconfirmed	B*52:20	Unconfirmed	B*52:49N	Unconfirmed		
B*52:01:23	Confirmed	B*52:21	Confirmed	B*52:50	Unconfirmed		
B*52:01:24	Unconfirmed	B*52:22	Unconfirmed	B*52:51	Unconfirmed		
B*52:01:25	Unconfirmed	B*52:23	Unconfirmed	B*52:52	Unconfirmed		
B*52:01:26	Unconfirmed	B*52:24	Unconfirmed	B*52:53	Unconfirmed		
B*52:01:27	Unconfirmed	B*52:25	Confirmed	B*52:54	Unconfirmed		
B*52:01:28	Unconfirmed	B*52:26	Unconfirmed	B*52:55	Unconfirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2015-October-10, release 3.22.0, www.ebi.ac.uk/imgt/hla.

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RESOLUTION IN HOMO- AND HETEROZYGOTES

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

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SPECIFICITY TABLE

HLA-B*52 SSP subtyping

Specificities and sizes of the PCR products of the 20+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}
1⁵	95 bp	800 bp	*52:01:01:01-52:02:02, 52:04-52:09, 52:12-52:18, 52:20-52:27, 52:30-52:56	*07:218, 13:62, 14:01:01-14:40, 14:42, 14:44-14:45, 14:47-14:50, 15:37-15:38:02, 15:323, 15:335, 15:364, 15:368, 18:15, 18:19, 18:30, 18:57, 35:21, 35:96, 35:188, 40:28, 40:51, 40:174, 49:09, 51:01:01:01-51:01:53, 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, 51:82-51:92:02, 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, 51:158:01-51:174, 51:176-51:179, 51:182-51:193, 51:195, 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, 78:09, C*01:30, C*08:51, C*08:114, C*12:87, C*15:39
2	135 bp 235 bp	1070 bp	*52:04 *52:09	*08:52, 15:87, 51:185, 53:05, 53:16, 53:33, 57:60
3^{5,6}	95 bp	1070 bp	*52:01:01:01-52:01:28, 52:03-52:15, 52:17, 52:19-52:31:02, 52:33-52:56	*13:58, 15:202, 15:239, 18:12:01-18:12:02, 35:10, 35:13, 35:16, 35:28, 35:69, 35:80, 35:226, 35:270, 37:01:01-37:01:05, 37:01:07-37:05, 37:07, 37:09-37:27, 37:29-37:57, 40:185, 40:209, 40:308, 44:83, 44:134, 49:03, 53:17:01-53:17:02, 53:28, 53:38, 78:05-78:06
4	140 bp	800 bp	*52:02:01-52:02:02	*15:13:01-15:13:02, 15:16:01-15:17:05, 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, 15:356, 15:361-15:362, 51:104, 57:01:01-57:11, 57:13-57:15, 57:17-57:44, 57:46-57:50, 57:52-57:68, 57:70-57:80, 58:36
5	280 bp 145 bp 190 bp	1070 bp	*52:36 *52:21 *52:03, 52:11	*15:01:02, 15:277-15:278, 51:61:01-51:61:02, C*02:02:23, C*12:03:31 *07:02:11, 07:05:03, 15:01:02, 15:09, 15:243, 15:252, 15:277-15:278, 15:361, 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,

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6	225 bp 135 bp	1070 bp	*52:05 *52:03, 52:10:01- 52:10:03	51:146, 51:180, 55:01:04, 78:04, C*02:02:23, C*12:03:31 *35:01:10, 35:04:02, 35:251, 40:01:06, 51:05 ^w , 51:09:01-51:09:03, 51:19, 51:31, 51:40, 51:54 ^w , 51:73, 51:93, 51:108, 51:122, 51:139, 51:194, 78:04, 78:08 *44:62 ^w , 51:23 ^w , 51:108 ^w
	180 bp 225 bp 160 bp 215 bp		*52:15 *52:05 *52:13 *52:19	
7^{7,8}		1070 bp		*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	1070 bp	*52:20	*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, 53:36, 53:38
	500 bp		*52:25	*13:58, 35:60, 35:291, 49:03, 51:15, 51:62, 51:157, 56:45, 58:73
9⁵	100 bp	800 bp	*52:06:01, 52:14	*13:46, 15:137, 35:01:08, 35:01:15, 35:29:02, 35:235, 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, 45:02, 50:09, 51:01:33, 51:13:01-51:13:02, 51:92:01-51:92:02, 51:172, 55:13, 55:58, 56:22, 59:03, 73:01-73:02
10⁵	285 bp 95 bp 245 bp	1070 bp	*52:49N *52:18 *52:08	*07:78, 07:84, 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, 13:84- 13:85, 27:14, 27:81, 27:130, 39:50, 40:06:01:01-40:06:14, 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, 40:306-40:307, 40:311, 40:313, 40:317, 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, 59:09, 73:01-73:02, 78:08
11^{5,7}	85 bp	1070 bp	*52:06:01-52:06:03	*07:38, 08:02-08:03, 08:52, 08:78, 08:117, 08:126, 27:23, 37:21, 38:06-38:07, 40:13, 44:06, 44:232, 51:01:01:01-51:01:16, 51:01:18-51:06:03, 51:08:01-51:21, 51:23- 51:24:05, 51:26-51:46, 51:48-51:117, 51:119- 51:195, 53:01:01-53:08:02, 53:10-53:16, 53:18-53:27, 53:29-53:37, 53:39, 53:41, 59:01:01:01-59:07, 59:09
12⁵	225 bp 85 bp	1070 bp	*52:17 *52:01:01:01-52:05, 52:07-52:17, 52:19- 52:56	*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, 13:68-13:85, 15:24:01-15:24:02, 15:36, 15:43, 15:87, 15:157, 15:256, 15:339, 27:29, 37:01:01-37:01:08, 37:01:10-37:04:02,

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13	165 bp	800 bp	*52:01:01:01-52:06:03, 52:08-52:56	*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, 35:58-35:59:02, 35:61:01-35:72, 35:74-35:78, 35:80-35:86, 35:88-35:184, 35:186-35:278, 35:280-35:300, 48:02:01-48:02:03, 51:01:01:01-51:24:05, 51:26-51:41N, 51:43-51:46, 51:48-51:156, 51:158:01-51:163, 51:165-51:195, 53:01:01-53:41, 56:05:01-56:05:02, 56:21, 56:36, 58:01:01:01-58:02, 58:04-58:10N, 58:12-58:19, 58:21-58:29, 58:31N-58:73, 67:05, 78:01:01-78:09, 81:03, 83:01?
14	165 bp	1070 bp	*52:07	*07:02:01-07:57, 07:59-07:256, 08:01:01-08:05, 08:07-08:156, 13:01:01-13:04, 13:06-13:23, 13:25-13:60, 13:62-13:85, 14:01:01-14:50, 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, 15:306-15:374, 18:01:01:01-18:15, 18:17N-18:116, 27:01-27:05:30, 27:06-27:21, 27:23-27:145, 35:31, 35:35, 35:43:01, 35:46, 35:57, 35:60, 35:79, 35:87, 35:185, 35:279, 37:01:01-37:57, 38:01:01-38:58, 39:01:01:01-39:01:01:04, 39:01:03-39:20, 39:22-39:45, 39:47-39:107, 40:01:01-40:06:07, 40:06:09-40:16, 40:18-40:40, 40:42-40:235, 40:237-40:271, 40:273-40:317, 41:01:01-41:41, 42:01:01-42:02:01:02, 42:04-42:21, 44:02:01:01-44:65, 44:67-44:117, 44:119-44:234, 45:01:01-45:17, 46:01:01-46:64, 47:01:01:01-47:10, 48:01:01-48:01:06, 48:02:02-48:37, 49:01:01-49:14, 49:16-49:40, 50:01:01-50:02, 50:04-50:20, 50:31-50:43, 51:42, 51:157, 54:01:01-54:34, 55:01:01-55:05, 55:07-55:76, 56:01:01:01-56:04, 56:06-56:20:02, 56:22-56:49, 57:01:01-57:01:10, 57:01:12-57:80, 58:11, 58:20, 59:01:01:01-59:09, 67:01:01-67:04, 78:08, 81:01-81:02, 81:04N-81:08, 82:01-82:02:01, 82:03, 83:01?
15⁵	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, 15:362, 35:37, 35:235, 35:265, 40:44, 40:159, 41:21, 44:20, 44:47, 44:100, 44:197, 45:09,

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				46:32, 49:04:01-49:05, 50:33, 51:37, 51:90, 51:92:01-51:92:02, 54:14-54:15, 55:23, 55:27, 55:68
16⁵	90 bp 120 bp	1070 bp	*52:31:01-52:31:02	*35:01:10, 35:04:02, 40:28, 44:62, 51:04, 51:46, 51:56:01-51:56:03, 51:139, 53:28
17	240 bp	1070 bp	*52:43	*51:65
18	160 bp	1070 bp	*52:30	*51:69
19⁵	65 bp	1070 bp	*52:53	
20	170 bp	800 bp	*52:16, 52:27	*07:27, 07:50, 08:04, 08:17, 08:54, 08:110, 15:03:01:01-15:03:05, 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, 15:369, 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, 18:103-18:106, 18:108-18:116, 37:01:01-37:20, 37:23-37:25, 37:27-37:56, 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, 39:101, 39:105, 40:12, 40:149, 41:22, 42:11, 44:130, 44:156, 44:221, 44:224, 48:01:01-48:02:01, 48:02:03-48:05, 48:07- 48:30, 48:32-48:37, 49:25
21⁹	-	-	-	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*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 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

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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 many non-HLA-B*52 alleles will be amplified by primer mixes 1 to 18 and 20.

In addition, a few HLA-C alleles will be amplified by primer mixes 1, 5 and 12.

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

⁶Primer mix 3 may give rise to a lower yield of HLA-specific PCR product than the other B*52 primer mixes.

⁷Primer mixes 7 and 11 may have tendencies of unspecific amplifications.

⁸Primer mix 7 has a tendency to giving rise to primer oligomer formation.

⁹Primer mix 21 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.

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

‘w’, might be weakly amplified.

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	95	135	95	140	145	135	160	145	100	95	85	85
		235		280	190	180	215	500	285	245	225	
					225	225						
Length of int. pos. control ¹	800	1070	1070	800	1070	1070	1070	1070	800	1070	1070	1070
5'-primer(s) ²	527 5'-TgA 3'	106 5'-CCA 3'	206 5'-gAC 3'	209 5'-ggC 3'	134 5'-CCC 3'	134 5'-CCC 3'	86 5'-CAA 3'	206 5'-gAC 3'	213 5'-CCg 3'	206 5'-gAC 3'	133 5'-CCA 3'	272 5'-CTC 3'
				652 5'-CCg 3'	435 5'-AAA 3'	435 5'-AAA 3'	362 5'-gAC 3'		357 5'-Tgg 3'	357 5'-Tgg 3'	272 5'-CTT 3'	
3'-primer(s) ³	583 5'-gTg 3'	200 5'-TCT 3'	259 5'-CTC 3'	309 5'-ATC 3'	317 5'-ggA 3'	317 5'-ggA 3'	206 5'-CCg 3'	309 5'-gTg 3'	272 5'-TgA 3'	262 5'-TgC 3'	317 5'-ggA 3'	317 5'-ggA 3'
		302 5'-ggC 3'		891 5'-CAg 3'	538 5'-CCA 3'	527 5'-CCA 3'	538 5'-gTC 3'	420 5'-gCT 3'	419 5'-CgA 3'	559 5'-CTC 3'		317 5'-ggA 3'
					583 5'-gTA 3'	527 5'-CCA 3'			601 5'-CTA 3'			
						572 5'-gCg 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
Length of spec. PCR product	165	165	95	90	240	160	65	170
				120				
Length of int. pos. control ¹	800	1070	800	1070	1070	1070	1070	800
5'-primer(s) ²	652 5'-CCg 3'	652 5'-CCA 3'	357 5'-Tgg 3'	355 5'-TCA 3'	383 5'-ggA 3'	357 5'-Tgg 3'	961 5'-TTA 3'	142 5'-TCT 3'
				385 5'-ggT 3'				
3'-primer(s) ³	774 5'-ggT 3'	774 5'-ggT 3'	412 5'-gTC 3'	435 5'-TCT 3'	583 5'-gTg 3'	475 5'-CgT 3'	985 5'-AgT 3'	272 5'-Tgg 3'
Well No.	13	14	15	16	17	18	19	20

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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CELL LINE VALIDATION SHEET								
HLA-B*52 SSP primer set ²								
					Well ³			
					17	18	19	20
				Prod. No.:	201562217	201562218	201562219	201562220
	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	-	-	-	-

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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.

³The A*34:0 allele is amplified by primer mix 12 in the IHL cell line.

No DNAs carrying the alleles to be amplified by primer solutions 2, 6 to 8 and 16 to 19 were available. The specificities of the primers in primer solutions 2, 6 to 8 and 16 were tested by separately adding one or two additional 5'-primers, respectively one or two additional 3'-primers. In primer solution 18 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 17 and 19 it were only possible to test the 3'-primers, the 5'-primers were not possible to test.

In primer solutions 5 to 7, 9 and 11 one or two 5'-primers were not possible to test, and in primer solutions 2, 9, 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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