

101.568-06 – including *Taq* polymerase, IFU-01
101.568-06u – without *Taq* polymerase, IFU-02

Visit <https://labproducts.caredx.com> for
“Instructions for Use” (IFU)

Lot No.: **6K7**

Lot-specific information

Olerup SSP[®] HLA-B*58

Product number:	101.568-06 – including <i>Taq</i> polymerase 101.568-06u – without <i>Taq</i> polymerase
Lot number:	6K7
Expiry date:	2024-01-01
Number of tests:	6
Number of wells per test:	31+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. 6K7.

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*58 Lot (7F4)

- The product documentation has been updated for new alleles of IMGT 3.38.0
- The kit resolution focuses on common and well documented (CWD) alleles¹.

¹As described in section Uniquely Identified Alleles.

The HLA-B*58 primer set, specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP[®]* HLA-B*58 lot was made (**Lot No. 7F4**).

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

Well	5'-primer	3'-primer	rationale
1	Added	-	3'-primer added for the B*58:111 allele.
30	-	Added	3'-primer added for the B*58:100 allele, 3'-primer added for improved yield of the B*58:62 allele.

¹S. J. Mack, P. Cano, J. A. Hollenbach et al.
Common and well-documented HLA alleles: 2012 update to
the CWD catalogue. Tissue Antigens, 2013, 81, 194–203

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Well **32** contains Negative Control primer pairs, that will amplify a majority 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 200 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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PRODUCT DESCRIPTION

HLA-B*58 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*58:01 to B*58:120 alleles.

PLATE LAYOUT

Each test consists of 32 PCR reactions in a 32 well 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	NC

The 32 well cut PCR plate is marked with 'HLA-B*58' in silver/gray ink.

Well No. 1 is marked with the Lot No. '6K7'.

Wells 1 to 31 – HLA-B*58 high resolution primers.

Well 32 – 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*58 alleles will be amplified by some primer mixes. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*58 alleles, i.e. **B*58:01 to B*58:120** recognized by the HLA Nomenclature Committee in October 2019^{1,2} will be amplified by the primers in the HLA-B*58 subtyping kit.

The HLA-B*58 kit enables separation of the confirmed HLA-B*58 alleles as listed in the IMGT/HLA database 3.25.0. 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*58 alleles is listed below.

The HLA-B*58 kit also enables identification of many null and alternatively expressed alleles.

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Lot-specific information

The following HLA-B*58 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix
B*58:10N, 58:13	12
B*58:21, 58:29	20
B*58:58, 58:66	26

¹HLA-B alleles listed on the IMGT/HLA web page 2019-October-17, release 3.38.0, 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*58:01:01:01	Confirmed	B*58:09	Unconfirmed	B*58:38	Unconfirmed	B*58:66	Unconfirmed
B*58:01:01:02	Unconfirmed	B*58:10N	Unconfirmed	B*58:39N	Unconfirmed	B*58:67	Unconfirmed
B*58:01:01:03	Unconfirmed	B*58:11	Confirmed	B*58:40	Confirmed	B*58:68	Unconfirmed
B*58:01:02	Unconfirmed	B*58:12	Confirmed	B*58:41	Unconfirmed	B*58:69	Unconfirmed
B*58:01:03	Unconfirmed	B*58:13	Unconfirmed	B*58:42	Unconfirmed	B*58:70	Unconfirmed
B*58:01:04	Unconfirmed	B*58:14	Unconfirmed	B*58:43	Unconfirmed	B*58:71	Unconfirmed
B*58:01:05	Unconfirmed	B*58:15	Unconfirmed	B*58:44	Unconfirmed	B*58:72N	Unconfirmed
B*58:01:06	Unconfirmed	B*58:16:01	Unconfirmed	B*58:45:01	Unconfirmed	B*58:73	Unconfirmed
B*58:01:07	Unconfirmed	B*58:16:02	Unconfirmed	B*58:45:02	Unconfirmed	B*58:74	Unconfirmed
B*58:01:08	Confirmed	B*58:17N	Unconfirmed	B*58:46	Unconfirmed	B*58:75	Unconfirmed
B*58:01:09	Unconfirmed	B*58:18	Confirmed	B*58:47	Unconfirmed	B*58:76	Unconfirmed
B*58:01:10	Unconfirmed	B*58:19	Unconfirmed	B*58:48	Unconfirmed	B*58:77	Unconfirmed
B*58:01:11	Unconfirmed	B*58:20	Unconfirmed	B*58:49	Unconfirmed	B*58:78	Unconfirmed
B*58:01:12	Unconfirmed	B*58:21	Confirmed	B*58:50	Unconfirmed	B*58:79	Unconfirmed
B*58:01:13	Unconfirmed	B*58:22	Unconfirmed	B*58:51	Unconfirmed	B*58:80	Unconfirmed
B*58:01:14	Unconfirmed	B*58:23	Confirmed	B*58:52	Unconfirmed	B*58:81	Unconfirmed
B*58:01:15	Unconfirmed	B*58:24	Confirmed	B*58:53	Unconfirmed		
B*58:01:16	Unconfirmed	B*58:25	Unconfirmed	B*58:54	Unconfirmed		
B*58:01:17	Unconfirmed	B*58:26	Confirmed	B*58:55	Unconfirmed		
B*58:01:18	Unconfirmed	B*58:27	Unconfirmed	B*58:56	Unconfirmed		
B*58:01:19	Unconfirmed	B*58:28:01	Unconfirmed	B*58:57	Unconfirmed		
B*58:01:20	Unconfirmed	B*58:28:02	Confirmed	B*58:58	Unconfirmed		
B*58:02:01	Confirmed	B*58:29	Unconfirmed	B*58:59:01	Confirmed		
B*58:02:02	Unconfirmed	B*58:31N	Unconfirmed	B*58:59:02	Unconfirmed		
B*58:04	Confirmed	B*58:32	Unconfirmed	B*58:60	Unconfirmed		
B*58:05	Unconfirmed	B*58:33	Unconfirmed	B*58:61	Unconfirmed		
B*58:06	Confirmed	B*58:34	Unconfirmed	B*58:62	Confirmed		
B*58:07	Confirmed	B*58:35	Unconfirmed	B*58:63	Unconfirmed		
B*58:08:01	Unconfirmed	B*58:36	Unconfirmed	B*58:64	Unconfirmed		
B*58:08:02	Confirmed	B*58:37	Unconfirmed	B*58:65	Unconfirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2016-July-14, release 3.25.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-B*58 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

HLA-B*58 SSP subtyping

Specificities and sizes of the PCR products of the 31+1 primer mixes used for HLA-B*58 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*58 alleles ³	Other amplified HLA-B alleles
1^{4,5}	90 bp	800 bp	*58:01:01:01-58:02:02, 58:05-58:29, 58:31N-58:35, 58:37-58:38, 58:40-58:43, 58:45:01-58:99, 58:101-58:120	*57:45, 57:51, 57:69, 57:120
2	150 bp	800 bp	*58:01:01:01-58:01:34, 58:04-58:05, 58:09-58:15, 58:17N, 58:19, 58:21-58:24, 58:28:01-58:29, 58:31N-58:37, 58:39N-58:42, 58:44-58:45:02, 58:47-58:59:02, 58:62-58:103, 58:105-58:120	*07:235, 07:359, 08:49, 13:01:01:01-13:01:16, 13:06-13:07N, 13:12:01-13:13:02, 13:17, 13:20, 13:22:01-13:23, 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, 13:86-13:87, 13:90, 13:92, 13:95, 13:106-13:107, 13:109-13:110, 13:122, 13:129, 13:135, 13:138, 14:10, 15:02:01:01-15:02:12, 15:13:01-15:13:02, 15:20-15:21:01:02, 15:25:01:01-15:25:04, 15:36, 15:44, 15:62, 15:77, 15:80, 15:85, 15:88-15:89:02, 15:106, 15:112, 15:121, 15:139, 15:144, 15:154, 15:165, 15:170, 15:194, 15:204, 15:213-15:214, 15:223, 15:240, 15:250, 15:265, 15:271, 15:283, 15:289, 15:291, 15:297, 15:301-15:302N, 15:308, 15:319, 15:325, 15:328, 15:330, 15:341, 15:345, 15:357-15:358, 15:366, 15:374, 15:378, 15:393-15:394, 15:399, 15:402, 15:404, 15:407, 15:418, 15:420, 15:425, 15:437-15:438, 15:454N, 15:458, 15:460, 15:463N, 15:468, 15:491, 15:495, 15:505, 15:537, 15:543-15:544N, 18:22, 18:69, 18:105, 18:149-18:150, 27:19:01:01-27:19:01:02, 27:30, 27:127, 27:150, 35:01:01:01-35:01:54, 35:01:56-35:01:61, 35:01:63-35:04:03, 35:06-35:08:13, 35:10-35:17:03, 35:19-35:21, 35:23-35:30:01:02, 35:33-35:36, 35:38-35:42:02, 35:45-35:50, 35:52, 35:54-35:57, 35:59:01-35:59:02, 35:61:01-35:63, 35:65Q, 35:69-35:71, 35:74, 35:76-35:78, 35:80-35:85, 35:90-35:96, 35:98, 35:100-35:101:02, 35:103-35:113, 35:115-35:116, 35:120-35:126, 35:128-35:134N, 35:136-35:150:02, 35:152-35:173:02N, 35:175-35:184, 35:186-35:198, 35:200-35:204, 35:206-35:229, 35:231, 35:233, 35:236-35:250, 35:252-35:259, 35:261-35:264, 35:266-35:272, 35:274-35:275, 35:277-35:291, 35:293-35:320, 35:322-35:330, 35:332-35:366, 35:370-35:371, 35:373-35:388, 35:390N-35:408, 35:410-35:415, 35:417-35:426, 35:428Q-35:432, 35:434-35:439:01:02, 35:441-35:458,

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3	240 bp	800 bp	*58:02:01:01-58:02:02, 58:06-58:07, 58:25, 58:38, 58:43, 58:46, 58:60, 58:104	*14:19, 14:27, 15:510, 44:440, 50:51, 57:11, C*01:35, C*01:107, C*03:17:01-03:17:02, C*03:71, C*03:249, C*03:271, C*03:302, C*06:96, C*06:197, C*12:122, C*14:20, C*16:149
4	190 bp 230 bp	1070 bp	*58:04 *58:15	*51:258
5⁴	90 bp	1070 bp	*58:05	*15:124, 15:507, 15:519, 18:88, 38:43, 40:77, 40:87:01-40:87:02, 40:222, 40:237, 40:368, 40:422, 44:247, 46:43, 48:14, 48:26, 48:29, 49:29, 51:181, 52:74, 57:59, C*07:81, C*07:168, C*07:450
6	170 bp	1070 bp	*58:06, 58:08:01-58:08:02, 58:19, 58:46, 58:78, 58:91	*07:02:01:01-07:24, 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, 07:236-07:260, 07:262-07:300, 07:302-07:331, 07:333-07:345, 07:347-07:358, 07:360Q-07:362, 07:364-07:385, 07:387-07:395, 08:20:01-08:20:02, 08:53:01-08:53:02, 08:79, 08:156, 13:16, 13:20, 13:31, 13:48, 13:62, 13:128, 13:131, 14:01:01:01-14:40, 14:42, 14:44-14:55, 14:57-14:58, 14:60-14:86, 15:01:01:01-15:01:04, 15:01:06-15:04:04, 15:06-15:17:04, 15:17:06-15:19, 15:21:01:01-15:21:01:02, 15:23-15:30:01:03, 15:32:01-15:40:02, 15:42-15:47:02, 15:49-15:50, 15:53-15:54, 15:56-15:58, 15:60-15:74, 15:76-15:82, 15:85, 15:87, 15:89:01-15:90, 15:92-15:99, 15:101-15:104, 15:106, 15:108-15:110, 15:112-15:113, 15:115-15:122, 15:125:01-15:129, 15:131-15:135, 15:137-15:144, 15:146-15:150, 15:152-15:154, 15:156-15:161, 15:163-15:175,

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7 ⁴	80 bp	800 bp	*58:06, 58:08:01-58:08:02, 58:19, 58:46, 58:91	56:05:01-56:06, 56:21, 56:25, 56:32, 56:37, 56:66, 57:09, 57:13, 57:22, 57:57, 57:63, 78:01:01:01-78:03, 78:05-78:07, 78:09
				*07:02:35 ^w , 07:68:02 ^w , 07:262 ^w , 08:20:02 ^w , 13:16, 13:20, 13:48, 13:62, 14:01:01:01-14:02:06, 14:02:08-14:11, 14:12 ^w , 14:13-14:42, 14:44-14:46, 14:47 ^w , 14:48-14:55, 14:57-14:58, 14:60-14:86, 15:02:09, 15:03:02, 15:03:04, 15:13:02, 15:39:02, 15:42, 15:435, 18:15, 18:57:02, 18:152, 27:153, 35:11:01, 35:11:03 ^w , 35:21, 35:58, 35:96, 35:167, 35:188, 35:277, 35:337:02 ^w , 35:384, 35:439:01:01-35:439:01:02, 38:10, 38:32, 39:35, 39:107, 40:28, 40:95, 42:19, 44:76 ^w , 44:79:01, 44:459, 48:25, 49:01:01:01-49:01:01:09, 49:01:03-49:01:13, 49:01:15-49:10, 49:12-49:14, 49:16-49:21, 49:23-49:40, 49:41 ^w , 49:42-49:44, 49:46-49:65, 50:01:01:01-50:02:01:02, 50:04-50:20, 50:31, 50:32 ^w , 50:33-50:46, 50:48, 50:50-50:65, 50:67-50:69, 51:01:01:01-51:01:24, 51:01:26-51:01:36, 51:01:38-51:01:46, 51:01:47 ^w , 51:01:48-51:01:51, 51:01:53-51:02:04, 51:02:06-51:04:02, 51:06:01-51:07:02, 51:11N-51:14, 51:16-51:18, 51:21-51:24:01, 51:24:04-51:24:05, 51:26-51:28, 51:29 ^w , 51:30, 51:32-51:35, 51:37-51:39, 51:41N, 51:43, 51:45-51:46, 51:48-51:53, 51:55 ^w , 51:56:01-51:60, 51:62-51:70, 51:74-51:80, 51:82 ^w , 51:83-51:86, 51:88-51:90, 51:92:01-51:92:02, 51:94-51:96, 51:98N-51:107, 51:109-51:114, 51:116-51:121, 51:123-51:125, 51:127-51:138, 51:140, 51:142-51:147, 51:149N-51:152, 51:154-51:156, 51:158:01-51:173Q, 51:174:02-51:180, 51:182-51:193, 51:195-51:196, 51:198-51:210, 51:212-51:216, 51:218-51:219, 51:221-51:258, 51:260-51:262, 51:264N-51:273N, 51:277-51:278, 51:281-51:307, 52:01:01:01-52:01:06, 52:01:08-52:01:14, 52:01:16-52:01:25, 52:01:26 ^w , 52:01:27-52:02:02, 52:04-52:09:02, 52:11-52:18, 52:20, 52:22-52:23, 52:24 ^w , 52:25:01-52:43, 52:44 ^w , 52:45-52:53, 52:55-52:60, 52:61 ^w , 52:62-52:70, 52:72-52:73, 52:75-52:94N, 53:06, 53:08:01, 53:28, 54:20 ^w , 55:01:01:01-55:01:23, 55:03, 55:05, 55:09 ^w , 55:11, 55:14-55:15, 55:17, 55:24-55:25, 55:28-55:29, 55:31, 55:33, 55:36, 55:38, 55:40, 55:44-55:45, 55:52 ^w , 55:53-55:55N, 55:58-55:60, 55:64, 55:66, 55:68, 55:73-55:76, 55:78-55:79, 55:84-55:85, 55:87, 55:90-55:95, 55:97N-55:106, 56:05:01-56:06, 56:21, 56:25, 56:32, 56:37, 56:66, 78:01:01:01-78:03, 78:05-78:07, 78:09
8	250 bp	1070 bp	*58:07	*44:440, C*06:165, C*14:61
9 ^{4,5}	90 bp	800 bp	*58:44	*07:219
	160 bp 190 bp		*58:08:01, 58:23 *58:72N, 58:74	*57:01:20, A*02:01:33
10 ⁴	115 bp	800 bp	*58:24	C*03:141, C*03:233, C*15:18
	270 bp		*58:09, 58:76	*14:10, 18:22, 18:69, 18:105, 18:149-18:150, 35:21, 35:24:01-35:24:02, 35:81, 35:96, 35:109,

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11	210 bp	1070 bp	*58:02:01:01-58:02:02, 58:07, 58:25, 58:38, 58:43, 58:60, 58:104	*14:43, 14:59, 18:44:01-18:44:02, 40:243, 44:440, 54:38, 57:11, A*24:64^w, C*01:06^w, C*06:79N, C*06:153, C*12:175, C*14:02:32, C*14:53, C*14:71^w, C*16:43^w
12	145 bp 235 bp	1070 bp	*58:13 *58:10N	*35:419, 49:06, 51:283
13	165 bp	1070 bp	*58:01:01:01-58:02:02, 58:04-58:10N, 58:12-58:19, 58:21-58:29, 58:31N-58:104, 58:107-58:120	*15:20, 15:228, 15:305, 15:435, 27:197, 35:01:01:01-35:01:27, 35:01:29-35:30:01:02, 35:32:01-35:32:02, 35:33-35:34, 35:36-35:42:02, 35:45, 35:47-35:56, 35:58-35:59:02, 35:61:01-35:72, 35:74-35:78, 35:80-35:86, 35:88-35:134N, 35:136-35:184, 35:186-35:208:01, 35:209-35:278, 35:280-35:358, 35:360-35:466, 37:80, 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, 51:165-51:219, 51:221-51:236, 51:238-51:294, 51:296-51:299, 51:301-51:306N, 52:01:01:01-52:06:03, 52:08-52:94N, 53:01:01:01-53:58, 56:05:01-56:05:02, 56:21, 56:36, 67:05, 78:01:01:01-78:10, 81:03
14⁶	165 bp	1070 bp	*58:11, 58:20	*07:02:01:01-07:57, 07:59-07:395, 08:01:01:01-08:05, 08:07-08:190, 08:192-08:249, 13:01:01:01-13:01:11, 13:01:13-13:02:28, 13:02:30-13:04, 13:06-13:23, 13:25-13:60, 13:62-13:139N, 14:01:01:01-14:86, 15:01:01:01-15:01:04, 15:01:06-15:19, 15:21:01:01-15:21:01:02, 15:23-15:40:02, 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, 15:306-15:434, 15:436-15:556, 18:01:01:01-18:15, 18:17N-18:189, 27:01-27:05:30, 27:05:32-27:21, 27:23-27:196, 27:198-27:207, 27:209-27:221, 35:31, 35:32:03, 35:35, 35:43:01, 35:43:03-35:44, 35:46, 35:57, 35:60, 35:79, 35:87, 35:135, 35:185, 35:208:02, 35:279, 37:01:01:01-37:79N, 37:81-37:91, 38:01:01:01-38:86, 38:156-38:162, 39:01:01:01-39:01:01:12, 39:01:03:01-39:20, 39:22-39:45, 39:47-39:160, 40:01:01-40:01:60, 40:01:62-40:06:07, 40:06:09-40:16:01:02, 40:18-40:40, 40:42-40:235, 40:237-40:271, 40:273-40:417, 40:419-40:449, 41:01:01:01-41:63, 42:01:01:01-42:02:02, 42:04-42:24, 42:26-42:27, 44:02:01:01-44:65, 44:67-44:117, 44:119-44:245, 44:247-44:346, 44:437-44:460, 44:462-44:480Q, 45:01:01:01-45:25, 46:01:01:01-46:79N, 46:81, 47:01:01:02-47:10, 48:01:01:01-48:01:10, 48:02:02-48:50, 49:01:01:01-49:14, 49:16-49:63, 49:65, 50:01:01:01-50:02:01:02, 50:04-50:20, 50:31-50:69, 51:42, 51:157, 51:220, 51:237, 51:295, 51:300, 52:07, 54:01:01:01-54:41,

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15⁴	90 bp	1070 bp	*58:36	*15:462, 57:01:01:01-57:01:22, 57:01:24-57:04:01, 57:04:03-57:15, 57:17-57:19, 57:21-57:35, 57:37-57:44, 57:46-57:50, 57:52-57:61, 57:63-57:68, 57:70-57:88, 57:90-57:108, 57:110-57:119, 57:121-57:127
	155 bp		*58:12	*07:219, 57:58, 57:80, A*02:42:01-02:42:02 , 02:310
	190 bp		*58:74	
16⁴	100 bp	1070 bp	*58:27-58:28:02, 58:65	*07:05:01:01-07:06:03, 07:32, 07:34, 07:40, 07:53, 07:69, 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, 07:258, 07:262, 07:264, 07:269-07:270, 07:278, 07:283-07:284, 07:293, 07:304, 07:317, 07:324, 07:332-07:333, 07:340, 07:349, 07:352-07:353, 07:356-07:358, 07:368, 07:378, 07:387, 07:389, 07:395, 08:01:01:01-08:05, 08:08N, 08:10-08:11, 08:13, 08:15, 08:17-08:27, 08:29, 08:31-08:34, 08:36, 08:38:01-08:48, 08:50-08:68, 08:70-08:83, 08:85-08:86N, 08:88, 08:90-08:106, 08:108-08:110, 08:112-08:155, 08:157-08:180-08:183, 08:185-08:194, 08:196-08:199, 08:201-08:226, 08:228-08:231, 08:233-08:249, 13:13:01-13:13:02, 13:21, 13:71, 13:86, 13:108, 14:13, 14:53, 15:324, 15:329, 27:07:01-27:07:06, 27:11, 27:24, 27:33, 27:43, 27:164, 27:180, 27:187, 27:219, 35:02:01:01-35:02:15, 35:04:01:01-35:04:03, 35:09:01-35:09:03, 35:12:01:01-35:12:04, 35:18, 35:22:01:01-35:22:01:02, 35:31, 35:81, 35:83, 35:87-35:88, 35:95, 35:129N, 35:149, 35:154, 35:157, 35:162, 35:172, 35:182-35:184, 35:201, 35:211, 35:220, 35:230, 35:233, 35:258, 35:266, 35:270, 35:285, 35:309, 35:311, 35:316, 35:321, 35:323, 35:335, 35:339, 35:357, 35:361, 35:366, 35:372, 35:374, 35:377-35:379, 35:384, 35:387, 35:391, 35:396, 35:403, 35:410, 35:419, 35:425, 35:443, 35:463, 37:12, 37:84, 39:14:01:01-39:14:01:03, 40:02:01:01-40:02:15, 40:02:17-40:02:30, 40:04:01-40:05:01:03, 40:08, 40:13, 40:15-40:16:01:02, 40:19, 40:28-40:30, 40:34-40:35:02, 40:37, 40:39-40:40, 40:45, 40:50:01:01-40:50:01:02, 40:56-40:58, 40:64:01:01-40:64:02, 40:68, 40:78, 40:80, 40:82, 40:85, 40:89-40:91, 40:94, 40:97, 40:99, 40:104, 40:107, 40:111, 40:115, 40:119, 40:122, 40:129, 40:133Q, 40:137, 40:142N-40:145, 40:157, 40:160:01-40:160:02, 40:164, 40:169, 40:173-40:174, 40:176, 40:181, 40:189, 40:200-40:203, 40:205-40:206, 40:209, 40:211, 40:214, 40:219-40:220, 40:224, 40:226,

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	225 bp		*58:84	40:229, 40:232, 40:246, 40:248, 40:254-40:255, 40:266, 40:271, 40:274, 40:276, 40:283, 40:287, 40:289-40:293, 40:296-40:297, 40:302-40:305, 40:309, 40:320, 40:322, 40:331, 40:334-40:335, 40:337N, 40:343, 40:345N, 40:347, 40:356, 40:359, 40:369, 40:371, 40:375, 40:378, 40:384, 40:390, 40:399N, 40:405, 40:409-40:410, 40:412-40:413, 40:415, 40:420, 40:432, 40:435, 40:437-40:438N, 40:440-40:442, 40:444, 41:02:01:01-41:02:09, 41:10-41:11, 41:13, 41:15, 41:19, 41:23, 41:27, 41:31, 41:36, 41:38-41:43, 41:45N- 41:49, 41:52, 41:55, 41:58, 41:62, 42:01:01:01-42:02:02, 42:05:01-42:05:02, 42:07-42:08, 42:10-42:15, 42:17-42:19, 42:21-42:27, 44:62, 44:77, 44:82, 44:166, 44:184, 44:213, 48:01:01:01-48:01:10, 48:04:01:01-48:06, 48:09-48:13, 48:15-48:16, 48:18-48:20, 48:22, 48:24, 48:27-48:39, 48:41-48:45, 48:47-48:50, 51:04:01-51:04:02, 51:46, 51:56:01-51:56:03, 51:64, 51:81, 51:139, 51:148, 53:19, 53:36, 55:04, 55:49, 55:51, 55:86, 56:12, 57:02:01-57:03:05, 57:05, 57:07, 57:09, 57:12, 57:17, 57:39, 57:42, 57:46, 57:57, 57:63, 57:66, 57:70, 57:80, 57:84, 57:94, 57:96, 57:101, 57:124, 81:01:01:01-81:09, C*03:317, C*03:382, C*15:24 *08:55, , 15:265, 35:35, 37:40, 38:19, 38:20:01-38:20:02, 39:03:01:01-39:03:01:03, 39:14:01:01-39:14:01:03, 39:24:01-39:24:02, 39:29, 39:37,39:42, 39:76, 39:120, 39:144, 39:152, C*15:51
17⁵	225 bp	1070 bp	*58:22, 58:84	*07:12, 07:14, 07:18:01-07:18:02, 07:137, 07:162-07:163, 07:199, 07:225, 07:227, 07:248, 07:350, 13:73, 15:265, 35:08:01:01-35:08:13, 35:18, 35:35, 35:45, 35:61:01-35:61:02, 35:80, 35:100, 35:105, 35:142, 35:156, 35:158, 35:164, 35:172, 35:174, 35:176, 35:187, 35:192, 35:206, 35:225, 35:229, 35:262, 35:300-35:301, 35:328, 35:333Q, 35:337:01-35:338, 35:341-35:342, 35:346, 35:356, 35:386, 35:388, 35:411, 35:437, 35:444-35:445, 35:461N, 35:466, 37:39-37:40, 38:20:01-38:20:02, 39:42, 44:04, 44:28:01-44:28:02, 44:56N, 44:76, 44:150, 53:11, 53:16, 53:24, 53:55, 57:02:01-57:02:02, 57:04:01-57:05, 57:12-57:13, 57:19, 57:28N, 57:30, 57:42, 57:63, A*01:41, C*01:49:01-01:50, C*01:176, C*03:28, C*03:49, C*03:117, C*03:251, C*03:293, C*04:80, C*04:100, C*04:110, C*04:370, C*05:10, C*05:148, C*08:126, C*14:54, C*15:08:01-15:08:02, C*15:51, C*15:143, C*15:148
18⁴	70 bp 140 bp	1070 bp	*58:25 *58:17N	
19⁴	95 bp	800 bp	*58:18	*07:09:01-07:09:02, 07:11, 07:17, 07:237, 07:287, 08:28, 08:35, 08:37, 15:07:01:01-15:07:03, 15:55, 15:68, 15:126, 15:197:02, 15:207, 15:331, 15:360, 15:383, 15:405, 15:421, 15:431, 15:450, 15:524, 18:14, 35:05:01:01-35:05:03, 35:51, 35:58, 35:66,

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20⁴	85 bp 145 bp 175 bp 275 bp	1070 bp	*58:29 *58:39N *58:21, 58:29 *58:21	*18:51, 53:34 *50:62, 53:34
21	440 bp	1070 bp	*58:14	*35:208:01-35:208:02, 57:120
22	160 bp	1070 bp	*58:51	
23⁷	150 bp 260 bp	800 bp	*58:26 *58:31N, 58:52	*35:75, 39:106, 44:138Q *51:32
24	190 bp 280 bp	1070 bp	*58:72N *58:40	
25⁷	130 bp 260 bp 375 bp	1070 bp	*58:57 *58:52 *58:35	*51:32
26⁴	75 bp 170 bp 375 bp	1070 bp	*58:66 *58:58 *58:35	
27	590 bp	1070 bp	*58:08:01-58:09, 58:76	*57:14:01-57:14:02
28	365 bp	800 bp	*58:01:20, 58:16:01-58:16:02, 58:18	*57:82
29	385 bp	1070 bp	*58:08:01-58:08:02, 58:20, 58:27, 58:59:01-58:59:02, 58:114	*07:219, 15:17:01:01-15:17:07, 15:162, 15:168, 15:177, 15:196, 15:208, 15:216, 15:230, 15:268, 15:273, 15:356, 15:361, 15:396, 15:403, 15:411, 15:423-15:424, 15:446, 15:462, 15:500, 15:516, 15:523, 15:532, 15:546Q, 15:555, 57:01:01:01-57:01:09, 57:01:11-57:04:03, 57:06-57:66, 57:68-57:127
30⁴	85 bp 170 bp	1070 bp	*58:100 *58:62	
31	145 bp 550 bp	1070 bp	*58:39N *58:93N	
32⁸	-	-	-	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*58 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

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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.

⁴HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁵Primer mixes 1, 9 and 17 may have tendencies of primer oligomer formation.

⁶Primer mix 14 may have tendencies of unspecific amplifications.

⁷Primer mixes 23 and 25 may give rise to a lower yield of HLA-specific PCR product than the other B*58 primer mixes.

⁸Primer mix 32 contains a negative control, which will amplify a majority 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 200 base pairs.

Abbreviations

‘?’ , 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	90	150	240	190	90	170	80	250	90	115	210	145
				230					160	270		235
									190			
Length of int. pos. control ¹	800	800	800	1070	1070	1070	800	1070	800	800	1070	1070
5'-primer(s) ²	206	354	361	106	463	527	486	361	105	355	361	367
	5'-gAA 3'	5'-ATA 3'	5'-AgT 3'	5'-CCA 3'	5'-TgA 3'	5'-TgA 3'	5'-ACC 3'	5'-AgT 3'	5'-gCT 3'	5'-TCA 3'	5'-AgT 3'	5'-T.T 3'
	207	355							141			454
	5'-ACC 3'	5'-TCA 3'							5'-ATT 3'			5'-ACA 3'
	209								206			
	5'-ggA 3'								5'-AgA 3'			
									355			
									5'-TCA 3'			
3'-primer(s) ³	256	463	559	256	512	3 rd I	527	572	256	430	527	559
	5'-CCC 3'	5'-gCT 3'	5'-CAg 3'	5'-CTC 3'	5'-CCA 3'	5'-TAT 3'	5'-CCT 3'	5'-gCg 3'	5'-CCC 3'	5'-gCg 3'	5'-CCA 3'	5'-CAg 3'
	256			296					479	583	540	
	5'-CCC 3'			5'-CTg 3'					5'-CCA 3'	5'-gTg 3'	5'-CTT 3'	
									506			
									5'-TgC 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	165	165	90	100	225	70	95	85	440	160	150	190
			155	225		140		145			260	280
			190					175				
								275				
Length of int. pos. control ¹	1070	1070	1070	1070	1070	1070	800	1070	1070	1070	800	1070
5'-primer(s) ²	652	652	105	355	355	209	363	106	206	653	355	355
	5'-CCg 3'	5'-CCA 3'	5'-gCT 3'	5'-TCA 3'	5'-TCA 3'	5'-ggA 3'	5'-AgC 3'	5'-CCA 3'	5'-gAC 3'	5'-CgC 3'	5'-TCC 3'	5'-TCA 3'
	652		142	363				209			652	
	5'-CTg 3'		5'-TCT 3'	5'-AgC 3'				5'-ggA 3'			5'-CCg 3'	
			209									
			5'-ggC 3'									
3'-primer(s) ³	774	774	256	412	538	239	419	208	362	774	463	506
	5'-ggT 3'	5'-ggT 3'	5'-CCC 3'	5'-gTT 3'	5'-CCg 3'	5'-gCg 3'	5'-Cgg 3'	5'-CTA 3'	5'-TCA 3'	5'-ggT 3'	5'-gCT 3'	5'-TgC 3'
				544	544	309		252			870	596
				5'-ggT 3'	5'-ggT 3'	5'-A.C 3'		5'-TCg 3'			5'-gA 3'	5'-TCT 3'
								340			872	
								5'-ggT 3'			5'-TCA 3'	
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

101.568-06 – including *Taq* polymerase, IFU-01
101.568-06u – without *Taq* polymerase, IFU-02

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“Instructions for Use” (IFU)

Lot No.: **6K7**

Lot-specific information

Well No.	25	26	27	28	29	30	31
Length of spec.	130	75	590	365	385	85	145
PCR product	260	170				170	550
	375	375					
Length of int. pos. control ¹	1070	1070	1070	800	1070	1070	1070
5'-primer(s) ²	2 nd I	2 nd I	281	281	281	209	106
	5'-CCg 3'	5'-CCg 3'	5'-CTC 3'	5'-CTC 3'	5'-CTC 3'	5'-ggA 3'	5'-CCA 3'
	652	652					
	5'-CCg 3'	5'-CCg 3'					
3'-primer(s) ³	559	559	583	353	379	255	208
	5'-CgT 3'	5'-CgT 3'	5'-gTg 3'	5'-ggg 3'	5'-CAC 3'	5'-Cgg 3'	5'-CTA 3'
	739	685		369	379	339	369
	5'-gTA 3'	5'-ggA 3'		5'-CCg 3'	5'-CAC 3'	5'-gCg 3'	5'-CCT 3'
	872	782					
	5'-TCA 3'	5'-CTT 3'					
Well No.	25	26	27	28	29	30	31

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.

101.568-06 – including *Taq* polymerase, IFU-01
101.568-06u – without *Taq* polymerase, IFU-02

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Lot No.: **6K7**

Lot-specific information

[illegible]

101.568-06 – including *Taq* polymerase, IFU-01
101.568-06u – without *Taq* polymerase, IFU-02

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Lot No.: 6K7

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 4, 5, 8 to 10, 12, 18, 20 to 28, 30 and 31 were available. The specificities of the primers in primer solutions 5, 8 to 10, 23, 25 to 28 and 30 were tested by separately adding one or two additional 5'-primers, respectively one, two or three additional 3'-primers. In primer solutions 4, 18, 20, 21, 24 and 31 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 12 and 22 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 9 to 11, 23, 25 and 26 one or two 3'-primers were not possible to test. In primer solutions 1, 2, 13, 25 and 26 one 5'-primer was not possible to test. In addition, one or more 3'- and/or 5'-primers in primer solutions 1, 15 to 17 and 29 were tested by separately adding one additional 5'-primer or one additional 3'-primer.

101.568-06 – including *Taq* polymerase, IFU-01
101.568-06u – without *Taq* polymerase, IFU-02

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Lot-specific information

101.568-06 – including *Taq* polymerase, IFU-01
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Lot No.: **6K7**

Lot-specific information

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