

Lot No.: 2E2

Lot-specific information

Olerup SSP® HLA-C*01

Product number:	101.621-12 – including <i>Taq</i> polymerase 101.621-12u – without <i>Taq</i> polymerase
Lot number:	2E2
Expiry date:	2019-05-01
Number of tests:	12
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. 2E2.

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-C*01 LOT (37Y)**

The HLA-C*01 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

¹As described in section Uniquely Identified Alleles.

The HLA-C*01 specificity and interpretation tables have been updated for the HLA-C alleles described since the previous *Olerup SSP®* HLA-C*01 lot was made (**Lot No. 37Y**). The kit design is based on IMGT/HLA database 3.25.0.

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

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

Well	5'-primer	3'-primer	rationale
1	-	Added	3'-primer added for the C*01:90 allele.
3	-	Added	3'-primer added for the C*01:111N allele.
4	Moved	Moved	5'-primer moved to well 12, 3'-primers moved to wells 15 and 25 for decreased tendency of primer oligomer formation.
12	Added	-	5'-primer added from well 4 for the C*01:82 allele.
15	-	Added	3'-primer added from well 4 for the C*01:16 allele.
17	Modified, moved	Modified, moved	Primer pair modified and moved to wells 27 and 29 for improved HLA-specific amplification.
22	Moved	Moved	Primer pair for the C*01:49 allele moved to well 28.
23	-	Added	3'-primer added for the C*01:103 allele.
24	-	Added	3'-primer added for the C*01:103 allele.
25	-	Added	3'-primer added from well 4 for the C*01:16 allele.
26	-	Added	3'-primer added for the C*01:117N allele.
27	Modified, Added	Added	5'-primer modified for increased yield, primer pair added from well 17 for the C*01:59 and C*01:118 alleles.
28	Added	Added	Primer pair added from well 22 for the C*01:49 and C*01:50 alleles for improved HLA-specific amplification, 5'-primer added for the C*01:107 allele.
29	Added	Added	Primer pair added from well 17 for the C*01:59 and C*01:118 alleles, 3'-primer added for the C*01:109N allele.
30	-	Added	3'-primer added for the C*01:121Q allele.
31	-	Added	3'-primer added for the C*01:117N allele.

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Well **32** 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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PRODUCT DESCRIPTION

HLA-C*01 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the C*01:02 to C*01:124 alleles.

PLATE LAYOUT

Each HLA-C*01 test consists of 32 PCR reactions in a 32 well cut PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	NC

The 32 well PCR plate is marked with 'HLA-C*01' in silver/gray ink.

Well No. 1 is marked with the Lot No. '2E2'.

Wells 1 to 31 – HLA-C*01 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 Class I alleles some non-HLA-C*01 alleles will be amplified by primer mixes 1, 2, 4, 6 to 8, 10, 12 to 14, 16, 20, 22, 23, 27, 29 and 31. In addition, a few HLA-A and HLA-B alleles will be amplified by primer mixes 1, 4, 7 to 10, 12, 13, 15, 16 and 25.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-C*01 alleles, i.e. **C*01:02 to C*01:124**, recognized by the HLA Nomenclature Committee in July 2016^{1,2} will be amplified by the primers in the HLA-C*01 SSP kit³.

The HLA-C*01 kit enables separation of the confirmed HLA-C*01 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-C*01 alleles is listed below.

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The HLA-C*01 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-C*01 primer set cannot distinguish the following silent mutations: the C*01:02:01-01:02:40 alleles, the C*01:07:01-01:07:02 alleles, the C*01:12:01-01:12:02 and C*01:79:01-01:79:02 alleles or the C*01:32:01-01:32:02 alleles.

The following HLA-C*01 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
C*01:06, 01:38	5	C*01:29, 01:33	21
C*01:10, 01:52	9	C*01:31, 01:44	23
C*01:17, 01:41	14	C*01:32:01-01:32:02, 01:40	22
C*01:18, 01:42	15	C*01:70, 01:99	25
C*01:19, 01:43, 01:58	16	C*01:89, 01:109N	29
C*01:27, 01:45	19	C*01:93, 01:121Q	30
C*01:28, 01:56N	20		

¹HLA-C alleles listed on the IMGT/HLA web page 2016-July-14, release 3.25.0, 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-C*01 primer set cannot separate the C*01:120 from the B*54:18 allele. These alleles can be distinguished by the HLA-C low resolution kit.

The HLA-C*01 primer set cannot separate the C*01:123 from the C*03:86, 03:94 and 03:99 alleles. These alleles can be distinguished by the HLA-C low resolution kit and/or by the HLA-C*03 high resolution kit.

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ALLELE CONFIRMATION STATUS

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



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SPECIFICITY TABLE

HLA-C*01 SSP subtyping

Specificities and sizes of the PCR products of the 31+1 primer mixes used for HLA-C*01 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-C*01 alleles ³	Other amplified HLA Class I alleles ⁴
1^{5,7}	90 bp	800 bp	*01:02:01-01:02:40, 01:06-01:11, 01:13-01:20, 01:22-01:23, 01:25-01:33, 01:35, 01:38-01:48, 01:51-01:53, 01:56N-01:76, 01:80-01:85, 01:87-01:96, 01:98N-01:100, 01:103-01:113, 01:115-01:119, 01:121Q-01:124	*03:86, 03:94, 03:99, 03:302, 05:107, 06:179, 07:177, 15:37, 15:102, B*40:243
2⁵	90 bp 270 bp	1070 bp	*01:03, 01:24, 01:78 *01:15	*03:58, 04:37, 05:85, 07:364
3	150 bp	800 bp	*01:04, 01:111N	
4⁸	210 bp	800 bp	*01:05, 01:77	*04:240, 05:111, 07:37, 07:307, B*07:77, B*07:193
5⁵	105 bp 150 bp 200 bp	800 bp	*01:38 *01:20 *01:06	
6⁶	195 bp 230 bp	1070 bp	*01:07:01-01:07:02 *01:37N, 01:83	*06:43:01, 14:24:02 *14:35N
7^{5,6}	70 bp	1070 bp	*01:67	*03:03:10, 03:04:28, 04:01:11, 06:02:21, 07:02:36, 12:03:36, 16:01:19, A*01:01:33, A*02:01:29, A*03:01:42, A*11:01:40, A*24:07:02, A*26:01:09, A*32:01:09, A*33:01:07, A*68:01:06, B*07:02:21, B*13:02:03, B*15:01:39, B*27:05:06, B*35:08:07, B*40:01:10, B*40:02:11, B*44:02:37, B*44:03:08, B*51:01:24, B*73:01-73:02, B*82:02:02
	150 bp 195 bp		*01:20 *01:08	
8	210 bp	800 bp	*01:04, 01:09, 01:22, 01:35	*03:302, 06:23, 06:179, 07:177, 12:178, 15:37, B*40:243
9	160 bp 225 bp	800 bp	*01:52 *01:10, 01:83	B*40:243
10	210 bp 255 bp 290 bp	1070 bp	*01:22, 01:35 *01:30 *01:11	*03:302, 07:177, 15:37, B*40:243

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11	140 bp 345 bp	800 bp	*01:39 *01:12:01-01:12:02, 01:34, 01:79:01-01:79:02, 01:97, 01:101-01:102, 01:114	
12⁵	80 bp 155 bp	1070 bp	*01:84 *01:13	*03:213 *02:51, 03:87:01-03:87:02, 04:223, 05:09:01-05:09:03, 05:17, 05:52, 07:130, 08:15:01-08:15:02, 08:51, 12:144, 12:185, 16:27, B*15:33, B*15:248
13⁷	255 bp 155 bp	800 bp	*01:82 *01:02:01-01:66, 01:68- 01:72, 01:74-01:79:02, 01:81-01:116, 01:118- 01:122, 01:124	*07:316, 07:338, 15:104, B*54:18
14^{5,6}	120 bp 240 bp	1070 bp	*01:41 *01:17, 01:21, 01:23, 01:69N	*07:316, 07:338
15⁵	115 bp 230 bp	800 bp	*01:42, 01:73 *01:16, 01:18, 01:74	B*15:393, B*46:60, B*51:129
16	130 bp 255 bp 295 bp	1070 bp	*01:43 *01:19 *01:23, 01:58	*07:316, 07:338, A*01:24
17⁵	75 bp 255 bp	800 bp	*01:24-01:25 *01:82	
18⁶	195 bp 260 bp 345 bp	800 bp	*01:26 *01:34 *01:36, 01:49:01, 01:55	
19⁵	100 bp 265 bp	800 bp	*01:27 *01:30, 01:45	
20⁵	80 bp 110 bp 285 bp	800 bp	*01:84 *01:28 *01:56N	*03:213 *03:59, 03:123, 06:157
21⁵	125 bp 160 bp 245 bp	800 bp	*01:33 *01:80 *01:29, 01:69N	
22⁵	110 bp 250 bp 335 bp	1070 bp	*01:40 *01:32:01-01:32:02 *01:50	*06:110
23⁵	90 bp 120 bp 235 bp	1070 bp	*01:04, 01:54, 01:103 *01:44 *01:31, 01:35, 01:107	*06:23, 12:178, 14:45, 16:18 *03:302
24⁵	90 bp 165 bp	1070 bp	*01:86N, 01:103 *01:66	
25^{5,8}	85 bp 240 bp 270 bp	1070 bp	*01:99 *01:16 *01:70	A*24:112, B*51:129
26	155 bp 230 bp	1070 bp	*01:117N *01:74, 01:98N	
27	350 bp	1070 bp	*01:14, 01:59, 01:118, *01:85	*04:37, 05:85, 05:107, 06:23, 06:179, 15:37, 15:102 *08:22, 08:56, 08:102, 15:29, 15:87
28	545 bp 155 bp 325 bp 360 bp	800 bp	*01:35, 01:107 *01:81 *01:49:01-01:50	
29	135 bp	1070 bp	*01:109N	

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	295 bp		*01:89N
	350 bp		*01:14, 01:59, 01:118
			*04:37, 05:85, 05:107, 06:23, 06:179, 15:37, 15:102
30⁵	125 bp	1070 bp	*01:93
	235 bp		*01:121Q
31	155 bp	1070 bp	*01:117N
	265 bp		*01:14
			*15:104
32⁹	-	-	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-C*01 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 Class I alleles some non-HLA-C*01 alleles will be amplified by primer mixes 1, 2, 4, 6 to 8, 10, 12 to 14, 16, 20, 22, 23, 27, 29 and 31. In addition, a few HLA-A and HLA-B alleles will be amplified by primer mixes 1, 4, 7 to 10, 12, 13, 15, 16 and 25.

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

⁶Primer mixes 6, 7, 14 and 18 may have tendencies of unspecific amplifications.

⁷Primer mixes 1 and 13 may give rise to a lower yield of HLA-specific PCR product than the other HLA-C*01 primer mixes.

⁸Primer mix 25 has a tendency to giving rise to primer oligomer formation.

⁹Primer mix 32 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.

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	90	90	150	210	105	195	70	210	160	210	140	80
PCR product		270			150	230	150		225	255	345	155
					200		195			290		255
Length of int. pos. control ¹	800	1070	800	800	800	1070	1070	800	800	1070	800	1070
5'-primer(s) ²	368	368	368	368	368	363	89	368	368	368	101	485
	5'-gTg ^{3'}	5'-gTg ^{3'}	5'-gTg ^{3'}	5'-gTC ^{3'}	5'-gTg ^{3'}	5'-TgA ^{3'}	5'-gAg ^{3'}	5'-gTg ^{3'}	5'-gTg ^{3'}	5'-gTg ^{3'}	5'-CAT ^{3'}	5'-CAA ^{3'}
						406	368		667		368	632
						5'-gCA ^{3'}	5'-gTg ^{3'}		5'-AgA ^{3'}		5'-gTT ^{3'}	5'-gAg ^{3'}
						667					368	806
						5'-AgA ^{3'}					5'-gTA ^{3'}	5'-ggT ^{3'}
3'-primer(s) ³	419	419	470	538	430	559	117	538	488	539	201	601
	5'-CgT ^{3'}	5'-CgA ^{3'}	5'-TCT ^{3'}	5'-CCg ^{3'}	5'-gCT ^{3'}	5'-CgT ^{3'}	5'-CCA ^{3'}	5'-CCA ^{3'}	5'-CCA ^{3'}	5'-TCA ^{3'}	5'-CTC ^{3'}	5'-CTC ^{3'}
	420	595	477		479	846	479	539	559	583	3 rd I	846
	5'-gCT ^{3'}	5'-CCT ^{3'}	5'-gCA ^{3'}		5'-CCA ^{3'}	5'-CAC ^{3'}	5'-CCA ^{3'}	5'-TCA ^{3'}	5'-CTC ^{3'}	5'-gTg ^{3'}	5'-ATg ^{3'}	5'-CAC ^{3'}
					527		523		846	619		
					5'-CCA ^{3'}		5'-ACA ^{3'}		5'-CAC ^{3'}	5'-TTT ^{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.	155	120	115	130	75	195	100	80	125	110	90	90
PCR product		240	230	255	255	260	265	110	160	250	120	165
				295		345		285	245	335	235	
Length of int. pos. control ¹	800	1070	800	1070	800	800	800	800	800	1070	1070	1070
5'-primer(s) ²	89	89	89	89	632	89	368	89	89	74	368	368
	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAg ^{3'}	5'-gAA ^{3'}	5'-gTg ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-C ^{3'}	5'-gTg ^{3'}	5'-gTg ^{3'}
	89				806	368		530	368	379	3 rd I	
	5'-gAA ^{3'}				5'-ggA ^{3'}	5'-gTT ^{3'}		5'-ggT ^{3'}	5'-gTg ^{3'}	5'-ACg ^{3'}	5'-Cgg ^{3'}	
					818	369		806		463		
					5'-ggC ^{3'}	5'-TAC ^{3'}		5'-ggT ^{3'}		5'-TgA ^{3'}		
						453						
						5'-AAT ^{3'}						
3'-primer(s) ³	201	170	164	176	846	244	427	331	172	142	418	418
	5'-CTT ^{3'}	5'-Cgg ^{3'}	5'-gCA ^{3'}	5'-ACT ^{3'}	5'-CAC ^{3'}	5'-CTg ^{3'}	5'-gTA ^{3'}	5'-CTA ^{3'}	5'-CAT ^{3'}	5'-TgA ^{3'}	5'-gTg ^{3'}	5'-gTg ^{3'}
	201	289	165	301		3 rd I	583	601	209	3 rd I	419	419
	5'-CTC ^{3'}	5'-AgC ^{3'}	5'-Tgg ^{3'}	5'-gCA ^{3'}		5'-ATg ^{3'}	5'-gTg ^{3'}	5'-CTC ^{3'}	5'-gCC ^{3'}	5'-ATg ^{3'}	5'-Cgg ^{3'}	5'-gT ^{3'}
		295	274	341			601	846	295		560	493
		5'-TCA ^{3'}	5'-CTg ^{3'}	5'-CgT ^{3'}			5'-CTg ^{3'}	5'-CAC ^{3'}	5'-TCA ^{3'}		5'-ACA ^{3'}	5'-CTT ^{3'}
			278						573		671	
			5'-ggT ^{3'}						5'-AgA ^{3'}		5'-ggA ^{3'}	
			287									
			5'-TCg ^{3'}									
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

Lot No.: **2E2**

Lot-specific information

Well No.	25	26	27	28	29	30	31
Length of spec.	85	155	350	155	135	125	155
PCR product	240	230	545	325	295	235	265
	270			360	350		
Length of int.	1070	1070	1070	800	1070	1070	1070
pos. control ¹							
5'-primer(s) ²	89	89	302	355	302	368	89
	5'-gAA 3'	5'-gAA 3'	5'-gAA 3'	5'-TCA 3'	5'-gAA 3'	5'-gTg 3'	5'-gAA 3'
			972	388	3rd I		
			5'-CTA 3'	5'-CCA 3'	5'-Cgg 3'		
				560			
				5'-CCT 3'			
3'-primer(s) ³	131	203	361	3 rd I	361	454	203
	5'-ggT 3'	5'-CTC 3'	5'-CCA 3'	5'-ATg 3'	5'-CCA 3'	5'-CTg 3'	5'-CTC 3'
	287	274	1034		683	562	312
	5'-TCg 3'	5'-CTg 3'	5'-AgT 3'		5'-CCT 3'	5'-gCT 3'	5'-AgT 3'
	319	283			843		
	5'-gCg 3'	5'-gC 3'			5'-gTC 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.

Lot No.: **2E2**

Lot-specific information

CELL LINE VALIDATION SHEET																				
HLA-C*01 SSP primer set ²																				
				Well ³																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Prod. No.:	201670801	201431102	201670803	201670804	201431105	201431106	201431107	201431108	201431109	201431110	201670811	201670812	201551813	201431114	201670815	201431116
IHCW cell line ¹			C*																	
1	9001	SA	*07:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*07:01	*15:05	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
3	9011	E4181324	*12:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*03:04	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*06:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*03:04	*07:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9007	DEM	*04:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*01:02		+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
11	9051	PITOUT	*16:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*06:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*01:02		+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
14	9071	OLGA	*01:02	*03:04	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
15	9075	DKB	*03:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*03:03	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*01:02	*07:05	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
19	9038	BM16	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*03:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*01:02	*15:02	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
24	9035	JBUSH	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*08:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*07:04	*15:05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*05:01	*16:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*16:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*17:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019	DJCAF	*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*17:01	*17:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*03:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*03:03	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*07:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*02:02	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*01:02	*06:02	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
41	9055	H0301	*08:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*01:02		+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
43	9076	T7526	*01:02	*08:01	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
44	9057	TEM	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*06:02	*17:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*07:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*07:04	*15:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*04:01	*16:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Lot No.: **2E2**

Lot-specific information

CELL LINE VALIDATION SHEET																			
HLA-C*01 SSP primer set ²																			
				Well ³															
				17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
				Prod. No.:	201670817	201431118	201431119	201551820	201431121	201670822	201670823	201670824	201670825	201670826	201670827	201670828	201670829	201670830	201670831
IHCW cell line ¹			C*																
1	9001	SA	*07:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*07:01	*15:05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*12:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*03:04	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*06:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*03:04	*07:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9007	DEM	*04:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*01:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*16:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*06:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*01:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*01:02	*03:04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*03:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*03:03	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*01:02	*07:05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*03:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*01:02	*15:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*08:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*07:04	*15:05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*05:01	*16:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*16:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*17:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*17:01	*17:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*03:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*03:03	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*07:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*02:02	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*01:02	*06:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*08:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*01:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*01:02	*08:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*06:02	*17:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*07:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*07:04	*15:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*04:01	*16:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Lot No.: 2E2**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.

³The B*73:01 allele is weakly amplified by primer mix 7 in the 9280 (LK707) cell line.

No DNAs carrying the alleles to be amplified by primer solutions 2 to 6, 8 to 12, 14 to 26 and 28 to 31 were available. The specificity of the primers in primer solutions 2 to 5, 8 to 12, 14 to 16, 18 to 20, 22 to 25, 28, 29 and 31 were tested by adding additional 5'-primers respectively 3'-primers.

In primer solutions 6 and 17 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solutions 21, 26 and 30 it was only possible to test the 5'-primer, the 3'-primers were not possible to test.

In primer solutions 9, 11 to 13, 20, 22 and 28 one of the 5'-primers was not possible to test.

In primer solutions 2, 3, 5, 7, 9, 10, 14 to 16, 18 to 20, 23 to 25, 29 and 31 one to four of the 3'-primers were not possible to test. Additional primers in primer solutions 1, 7, 13 and 27 were tested by separately adding one 5'-primer and/or one 3'-primer.

Lot No.: **2E2**

Lot-specific information

Lot No.: **2E2**

Lot-specific information

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