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101.622-12 - including *Taq* **pol.**, IFU-01 **101.622-12u - without** *Taq* **pol.**, IFU-02

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Lot No.: 99V Lot-specific information

Olerup SSP® HLA-C*02

Product number: 101.622-12 - including *Tag* polymerase

101.622-12u - without *Taq* polymerase

Lot number: 99V

Expiry date: 2016-December-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. 99V.

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*02 Lot (95R)

The HLA-C*02 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.

The HLA-C*02 specificity and interpretation tables have been updated for the HLA-C alleles described since the previous *Olerup* SSP® HLA-C*02 lot was made **(Lot No. 95R)**.

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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¹As described in section Uniquely Identified Alleles.

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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
4	Added	Added	Primer pair added for the C*02:56 allele.
6	Added	-	5'-primer added for the C*02:64 allele.
13	-	Added	3'-primer added for the C*02:49 allele.
15	-	Added	3'-primer added for the C*02:71 allele.
20	Added	Added	Primer pair added for the C*02:65 allele, 3'-primers added for the C*02:71 and C*02:72 alleles.
21	Added	-	5'-primers added for the C*02:64 and C*02:67Q alleles.
22	Added	Added	Primer pair added for the C*02:56 allele.
23	-	Added	3'-primers added for the C*02:69 and C*02:70 alleles.
25	Added	-	5'-primer added for the C*02:60 allele.
26	-	Added	3'-primer added from well 32.
27	-	Added	3'-primer added from well 32.
28	Added	-	5'-primers added for the C*02:60 and C*02:67 alleles.
32	Removed	Moved	3'-primers moved to wells 26 and 27.

• LERUPSSP® HLA-C*02

101.622-12 - including *Taq* **pol.**, IFU-01 **101.622-12u - without** *Taq* **pol.**, IFU-02

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Well **32** contains <u>Negative Control primer pairs</u>, 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	105	200	105	80	75	80	85
product							
5'-primer ¹	164	340	440	45	45	43	36
•	5'-CAC3'	^{5'} -Agg ^{3'}	^{5'} -TTA3'	⁵ '-Tgg ³ '	^{5'} -Tgg ^{3'}	⁵ '-Tgg ³ '	5'-TAC3'
							36
							^{5'} -TAT ^{3'}
3'-primer ²	231	2 nd 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'-ACA3'
							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 codonnumbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

of the primer is given.

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.: 99V Lot-specific information

PRODUCT DESCRIPTION

HLA-C*02 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the C*02:02 to C*02:81 alleles.

PLATE LAYOUT

Each HLA-C*02 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*02' in silver/gray ink.

Well No. 1 is marked with the Lot No. '99V'.

Wells 1 to 31 – HLA-C*02 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-C alleles, non-HLA-C*02 alleles will be amplified by primer mixes 1 to 8, 10, 12 to 14, 16 to 18, 20 to 22, 24, 25, 28 to 30 and 31. In addition, a few HLA-A and HLA-B alleles will be amplified by primer mixes 3 to 5, 12, 16, 19, 20, 22, 25, 28 and 31.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-C*02 alleles, i.e. **C*02:02 to C*02:81**, recognized by the HLA Nomenclature Committee in April 2014^{1,2} will be amplified by the primers in the HLA-C*02 SSP kit.

The HLA-C*02 kit enables separation of the confirmed HLA-C*02 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*02 alleles is listed below.

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Lot No.: 99V **Lot-specific information**

The HLA-C*02 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 following HLA-C*02 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
C*02:05:02-02:05:03, 02:22	5	C*02:25Q, 02:30	21
C*02:15, 02:21	15	C*02:35, 02:69, 02:70	23
C*02:24, 02:65	20	C*02:37, 02:52N	28

The HLA-C*02 primer set cannot distinguish the following silent mutations: the C*02:02:01-02:02:03, 02:02:06-02:02:12 and 02:02:15-02:02:25 alleles, the C*02:02:05 and 02:02:14 alleles, the C*02:05:02-02:05:03 alleles or the C*02:26:01-02:26:03 alleles.

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¹HLA-C alleles listed on the IMGT/HLA web page 2014-April-14, release 3.16.0,

www.ebi.ac.uk/imgt/hla.

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.

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Lot No.: 99V Lot-specific information

ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
C*02:02:01	Unconfirmed	C*02:05:03	Unconfirmed	C*02:31	Confirmed	C*02:62	Unconfirmed
C*02:02:02:01	Confirmed	C*02:06	Confirmed	C*02:32	Confirmed	C*02:63	Unconfirmed
C*02:02:02	Unconfirmed	C*02:07	Confirmed	C*02:33	Unconfirmed	C*02:64	Confirmed
C*02:02:03	Confirmed	C*02:08	Unconfirmed	C*02:34	Confirmed	C*02:65	Confirmed
C*02:02:05	Unconfirmed	C*02:09	Unconfirmed	C*02:35	Unconfirmed	C*02:66	Unconfirmed
C*02:02:06	Unconfirmed	C*02:10	Confirmed	C*02:36	Unconfirmed	C*02:67Q	Confirmed
C*02:02:07	Confirmed	C*02:11	Unconfirmed	C*02:37	Confirmed	C*02:68	Unconfirmed
C*02:02:08	Confirmed	C*02:12	Unconfirmed	C*02:38N	Confirmed	C*02:69	Unconfirmed
C*02:02:09	Confirmed	C*02:13	Confirmed	C*02:39	Unconfirmed	C*02:70	Unconfirmed
C*02:02:10	Unconfirmed	C*02:14	Confirmed	C*02:40	Confirmed	C*02:71	Confirmed
C*02:02:11	Confirmed	C*02:15	Unconfirmed	C*02:42	Unconfirmed	C*02:72	Unconfirmed
C*02:02:12	Confirmed	C*02:16:01	Unconfirmed	C*02:43	Unconfirmed	C*02:73	Unconfirmed
C*02:02:13	Confirmed	C*02:16:02	Confirmed	C*02:44	Confirmed	C*02:74	Unconfirmed
C*02:02:14	Unconfirmed	C*02:17	Confirmed	C*02:45	Unconfirmed	C*02:75	Unconfirmed
C*02:02:15	Unconfirmed	C*02:18	Confirmed	C*02:46	Confirmed	C*02:76	Unconfirmed
C*02:02:16	Confirmed	C*02:19	Confirmed	C*02:47	Unconfirmed	C*02:77	Unconfirmed
C*02:02:17	Unconfirmed	C*02:20	Unconfirmed	C*02:48	Unconfirmed	C*02:78	Unconfirmed
C*02:02:18	Unconfirmed	C*02:21	Unconfirmed	C*02:49	Confirmed	C*02:79	Unconfirmed
C*02:02:19	Unconfirmed	C*02:22	Unconfirmed	C*02:50	Unconfirmed	C*02:80	Unconfirmed
C*02:02:20	Unconfirmed	C*02:23	Unconfirmed	C*02:51	Unconfirmed	C*02:81	Unconfirmed
C*02:02:21	Confirmed	C*02:24	Confirmed	C*02:52N	Unconfirmed		
C*02:02:22	Unconfirmed	C*02:25Q	Confirmed	C*02:53	Confirmed		
C*02:02:23	Unconfirmed	C*02:26:01	Confirmed	C*02:54	Unconfirmed		
C*02:02:24	Confirmed	C*02:26:02	Confirmed	C*02:55	Confirmed		
C*02:02:25	Unconfirmed	C*02:26:03	Confirmed	C*02:56	Confirmed		
C*02:02:26	Unconfirmed	C*02:27:01	Confirmed	C*02:57	Unconfirmed		
C*02:03	Unconfirmed	C*02:27:02	Confirmed	C*02:58	Unconfirmed		
C*02:04	Unconfirmed	C*02:28	Unconfirmed	C*02:59	Unconfirmed		
C*02:05:01	Unconfirmed	C*02:29	Unconfirmed	C*02:60	Confirmed		
C*02:05:02	Confirmed	C*02:30	Confirmed	C*02:61	Unconfirmed		

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

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-C*02 homo- and heterozygotes is available upon request.

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Lot-specific information SPECIFICITY TABLE

HLA-C*02 SSP subtyping

Specificities and sizes of the PCR products of the 31+1 primer mixes used for HLA-C*02 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-C*02 alleles ³	Other amplified HLA Class I alleles ⁴
1	250 bp	800 bp	*02:02:01-02:02:03, 02:02:05-02:02:26, 02:04- 02:15, 02:17, 02:19-02:31, 02:33-02:40, 02:42-02:71, 02:73-02:81	*01:04, 01:09, 01:21, 04:94:01-04:94:02, 05:08, 05:52, 05:89, 06:02:01:01-06:02:01:03, 06:02:03-06:03:02, 06:07-06:39, 06:41-06:78, 06:80-06:117, 06:119-06:131, 08:27, 08:29, 08:31, 12:02:01-12:08, 12:10:01-12:13, 12:15-12:17, 12:21-12:23, 12:25-12:82, 12:84N, 12:86-12:125, 14:16, 15:74, 16:04:01, 16:29, 16:33, 16:42, 16:55, 16:61, 16:66, 18:03
2 ⁵	95 bp	800 bp	*02:02:01-02:02:03, 02:02:06-02:02:12, 02:02:15- 02:02:26, 02:04, 02:05:02- 02:05:03, 02:07-02:09, 02:11, 02:13, 02:15, 02:19-02:27:01, 02:28-02:31, 02:34-02:40, 02:42-02:50, 02:52N-02:57, 02:59-02:71, 02:74-02:75, 02:77-02:81	*12:03:17
3 ⁵	100 bp	800 bp	*02:03, 02:16:02, 02:18, 02:31	*03:03:22, 03:04:34, B*27:34, B*35:01:30^w , B*40:02:07 , B*40:06:02 , B*57:01:16^w ,
_	135 bp		*02:20	B*57:03:02 ^w
4 ⁵	65 bp 150 bp	1070 bp	*02:56 *02:04	B*35:01:30, B*57:01:16, B*57:03:02 *12:115, 14:41
5	145 bp 240 bp	1070 bp	*02:22 *02:05:01-02:05:03, 02:17	*08:31, B*07:02:07 , B*27:05:15 *01:10, 06:08, 12:119, 14:25, 16:29, 16:50, 17:21, B*40:243
6	160 bp 210 bp	800 bp	*02:06, 02:47 *02:46, 02:64	*12:15, 15:74
7	130 bp	800 bp	*02:07	*16:10, 16:67
8 ⁵	70 bp	1070 bp	*02:08	*03:18:02, 03:64:01, 12:03:23, 15:10:02-15:10:03
	280 bp		*02:33	
9	200 bp	1070 bp	*02:02:01-02:02:03, 02:02:05-02:09, 02:11-02:13, 02:15-02:28, 02:30-02:40, 02:42-02:57, 02:59-02:81	

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	-01 NO 33 V		Lot-specific information	
10 ⁷	130 bp	1070 bp	*02:02:01-02:02:03, 02:02:05-02:02:12, 02:02:14- 02:02:25, 02:03-02:25Q, 02:27:01-02:38N, 02:40, 02:42-02:44, 02:46-02:81	*04:03:01-04:03:02, 04:06, 04:80, 04:107, 04:147, 04:160, 05:26, 15:11, 16:34
11 ⁵	90 bp 170 bp	800 bp	*02:18, 02:32 *02:09	
12	150 bp	1070 bp	*02:11, 02:14	*04:42, 05:43, 06:05 ^w , 07:02:09, 08:37, 12:16, 15:23, 15:63, 16:21, B*27:84
	230 bp		*02:17	*14:25
13 ⁶	225 bp 265 bp	1070 bp	*02:12, 02:27:01-02:27:02 *02:49, 02:75	*16:34 *04:03:01-04:03:02, 04:06, 04:80, 04:147, 04:160
14 ⁵	80 bp 115 bp	800 bp	*02:13 *02:43	*12:03:17
15	130 bp 190 bp	1070 bp	*02:21 *02:15, 02:71	
16 ^{6,7}	250 bp	1070 bp	*02:03, 02:16:01-02:16:02, 02:18	*01:22, 01:35, 04:04:01-04:04:02, 04:06, 04:13, 04:34, 04:58, 04:122, 04:160, 05:11, 05:17, 05:27, 05:68, 05:70, 05:79, 06:04, 06:118, 08:01:01-08:01:15, 08:03:01-08:04:03, 08:06, 08:08:01-08:11, 08:13-08:14, 08:16:01-08:16:02, 08:20-08:22, 08:24, 08:26N, 08:36N, 08:38-08:42, 08:44, 08:46, 08:50, 08:54, 08:56-08:61, 08:65-08:66, 08:72:01-08:72:02, 08:78-08:89N, 08:91, 08:93, 08:95-08:99, 08:101-08:102, 08:104-08:106, 12:14:01-12:14:02, 12:18:01-12:18:02, 12:20, 12:83, 14:06, 14:15, 14:53, 15:02:01-15:07, 15:09-15:13, 15:15-15:19, 15:21-15:24, 15:26-15:73, 15:76-15:83, 15:85-15:87, 16:35, 16:40, 16:48, 17:01:01:01-17:16, 17:18-17:24, B*58:02
17 ⁵	110 bp	1070 bp	*02:31, 02:43	*12:03:17
	160 bp	•	*02:23	
18	235 bp	800 bp	*02:26:01-02:26:03	*03:07, 03:10, 03:15, 03:29, 03:45, 03:163, 04:16, 04:42, 04:103, 05:01:01:01-05:01:25, 05:01:27, 05:03-05:12, 05:14-05:19, 05:21-05:25, 05:27-05:29:02, 05:31-05:77, 05:79-05:102, 06:02:01:01-06:02:01:03, 06:02:03-06:02:08, 06:02:10-06:02:20, 06:02:22-06:10, 06:12-06:17, 06:19-06:32, 06:34-06:81, 06:83-06:103, 06:105-06:118, 06:121-06:124, 06:126-06:131, 07:07, 07:09, 07:76:01-07:76:02, 07:315, 07:328, 08:10,

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LO	t No.: 99V		Lot-specific information	
				12:04:01-12:05, 12:09, 12:21, 12:33, 12:41, 12:60, 12:72, 15:02:01-15:06:03, 15:08-15:10:03, 15:12-15:13, 15:15-15:19, 15:21-15:24, 15:26-15:35, 15:37-15:42, 15:44-15:84Q, 15:86-15:87, 16:02:01-16:02:07, 16:02:09-16:02:12, 16:09, 16:12, 16:19, 16:25, 16:37, 16:46-16:48, 16:60, 16:63, 16:69-16:70, 17:01:01-17:01:08, 17:01:10-17:21, 17:23-17:24, 18:01-18:07N
19 ⁵	80 bp	1070 bp	*02:03, 02:28	B*27:34, B*35:01:30 ^w , B*40:02:07, B*40:06:02, B*57:01:16 ^w , B*57:03:02 ^w
20 ⁵	50 bp 180 bp 215 bp	1070 bp	*02:65 *02:24, 02:71 *02:72	*01:59, 03:130, 03:140, 03:243, 04:114, 05:20, 06:82, 07:49, 07:210, 07:238, 07:247, 12:54, 14:04, 14:64, 15:85, 16:57, A*68:46 B*07:113, B*08:64, B*40:192 B*07:52
21 ⁵	115 bp 210 bp	1070 bp	*02:30 *02:25Q, 02:64, 02:67Q	*15:19
22 ⁵	65 bp	1070 bp	*02:56 *02:34	B*35:01:30, B*57:01:16, B*57:03:02 *16:09
23 ⁵	85 bp 210 bp 390 bp	1070 bp	*02:70 *02:29, 02:69 *02:35	
24	325 bp	1070 bp	*02:06, 02:23, 02:36, 02:68	*01:90, 03:81, 03:175, 03:199, 03:245, 04:108, 06:89, 07:123, 07:173, 07:294, 12:15, 12:113, 15:02:01-15:02:17, 15:02:19-15:03, 15:05:01-15:13, 15:15-15:19, 15:21-15:24, 15:26-15:29, 15:31-15:39, 15:41-15:63, 15:67-15:75, 15:78-15:87, 16:20
25	160 bp	800 bp	*02:19, 02:23	*01:09, 03:21, 03:80, 03:142, 06:107, B*07:55, B*07:100 , B*15:45, B*15:63, B*15:248 , B*15:287
	215 bp		*02:60	B*07:55, B*07:100, B*08:70, B*15:07:01-15:07:03, B*15:45, B*15:68, B*15:126, B*15:207, B*46:12, B*48:19
26	140 bp	1070 bp	*02:39-02:40, 02:53	
27	140 bp	800 bp	*02:44-02:45, 02:53	
28 ^{5,8}	90 bp 210 bp	800 bp	*02:52N *02:37, 02:46, 02:60, 02:67Q	*06:81, B*07:55, B*07:100, B*08:70, B*15:07:01-15:07:03, B*15:45, B*15:68, B*15:126, B*15:207, B*46:12, B*48:19

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29	210 bp	1070 bp	*02:12, 02:49, 02:55	*04:03:01, 04:06, 04:80, 04:107, 04:147, 04:160
30 ^{5,7}	80 bp 270 bp	1070 bp	*02:38N *02:58	*01:35, 04:08, 04:34, 04:147, 05:27, 05:39, 06:96, 08:41, 12:83, 12:106, 12:122, 14:20, 15:15, 15:77, 17:07
31 ⁵	100 bp	1070 bp	*02:42	*01:21, 04:140, 04:166, 05:98, 06:05, 07:02:09, 08:14, 08:80, 08:103, 12:16, 15:63, B*67:02
32 ⁹			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*02 high resolution 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.

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-C alleles, non-HLA-C*02 alleles will be amplified by primer mixes 1 to 8, 10, 12 to 14, 16 to 18, 20 to 22, 24, 25, 28 to 30 and 31. In addition, a few HLA-A and HLA-B alleles will be amplified by primer mixes 3 to 5, 12, 16, 19, 20, 22, 25, 28 and 31.

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

⁶Primer mixes 13 and 16 may give a lower yield of HLA-specific PCR product than the other C*02 primer mixes.

Primer mixes 10, 16 and 30 may have tendencies of unspecific amplifications.

⁸The C*07 alleles might be faintly amplified by primer mix 28.

⁹Primer mix 32 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. 'w', might be weakly amplified.

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Lot No.: 99V

Lot-specific information

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	250	95	100	65	145	160	130	70	200	130	90	150
PCR product			135	150	240	210		280			170	230
Length of int.	800	800	800	1070	1070	800	800	1070	1070	1070	800	1070
pos. control ¹												
5'-primer(s) ²	2 nd I	486	486	92	361	364	2 nd I	105	703	113	486	97
	5' -CCA 3'	^{5'} -ACA ^{3'}	^{5'} -ACA ^{3'}	^{5'} -gTg ^{3'}	^{5'} -AgT ^{3'}	^{5'} -ggT ^{3'}	5' -CCA 3'	^{5'} -gCT ^{3'}	^{5'} -CTA ^{3'}	5' -CCA 3'	5' -ACA 3'	^{5'} -TCg ^{3'}
				463	453	370				118		368
				^{5'} -TgA ^{3'}	^{5'} -AAT ^{3'}	5' -ACT 3'				5' -CCA 3'		^{5'} -gTT ^{3'}
						420						449
						^{5'} -TTA ^{3'}						5' -CCA 3'
3'-primer(s) ³	538	538	538	201	559	538	418	134	861	201	527	201
. (,	5' -CCA 3'	5' -CCA 3'	5' -CAg 3'	5' -CTT 3'	5' -CTC 3'	5' -CCA 3'	5' -gTC 3'	^{5'} -AgC ^{3'}	^{5'} -TCg ^{3'}	5' -CTT 3'	5' -CCg 3'	5' -CTT 3'
			555	486				343			538	559
			^{5'} -CCg ^{3'}	^{5'} -gCT ^{3'}				^{5'} -g ^{3'}			^{5'} -CCg ^{3'}	5' -CTC 3'
			578								613	
			^{5'} -TgT ^{3'}								^{5'} -gCA ^{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.	225	80	130	250	110	235	80	50	115	65	85	325
PCR product	265	115	190		160			180	210	110	210	
								215			390	
Length of int.	1070	800	1070	1070	1070	800	1070	1070	1070	1070	1070	1070
pos. control ¹												
5'-primer(s) ²	118	486	113	2 nd I	486	118	486	302	125	244	322	409
	5' -CCA 3'	5' -ACA 3'	5' -CCA 3'	5' -CCA 3'	5' -ACA 3'	^{5'} -CCg ^{3'}	5' -ACA 3'	^{5'} -gAA ^{3'}	^{5'} -CgA ^{3'}	^{5'} -CgC ^{3'}	^{5'} -gCC ^{3'}	5' -ggC
			369			118		369	370	463	703	
			^{5'} -TAC ^{3'}			^{5'} -CCg ^{3'}		^{5'} -TAC ^{3'}	5' -ACT 3'	^{5'} -TgA ^{3'}	^{5'} -CTA ^{3'}	
									373			
									^{5'} -gCg ^{3'}			
									374			
									^{5'} -CTA ^{3'}			
3'-primer(s) ³	302	527	201	538	555	312	527	312	201	312	419	3 rd I
	^{5'} -ggC ^{3'}	5' -CCg 3'	5' -CTT 3'	^{5'} -CAg ^{3'}	5' -CCg 3'	^{5'} -AgT ^{3'}	5' -CCA 3'	^{5'} -Agg ^{3'}	5' -CTT 3'	^{5'} -AgT ^{3'}	^{5'} -Cgg ^{3'}	5' -CTC
	302	559	515		559			506	538	486	745	
	^{5'} -ggC ^{3'}	^{5'} -CgT ^{3'}	5' -CCA 3'		5' -CgT 3'			^{5'} -Tgg ^{3'}	5' -CCA 3'	^{5'} -gCT ^{3'}	^{5'} -AgC ^{3'}	
	341		518		603			515			865	
	^{5'} -CgT ^{3'}		5' -CCA 3'		^{5'} -TTg ^{3'}			5' -CCA 3'			5' -CCT 3'	
								545			871	
								^{5'} -AgA ^{3'}			^{5'} -CgA ^{3'}	
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

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Lot No.: 99V Lot-specific information

Well No.	25	26	27	28	29	30	31
Length of spec.	160	140	140	90	210	80	100
PCR product	215			210		270	
Length of int.	800	1070	800	800	1070	1070	1070
pos. control ¹							
5'-primer(s) ²	363	105	105	105	118	2 nd I	142
	^{5'} -AgC ^{3'}	^{5'} -gCT ^{3'}	^{5'} -gCT ^{3'}	^{5'} -gCT ^{3'}	5' -CCA 3'	5' -CCA 3'	5' -TCT 3'
	419	486	486	359			
	^{5'} -gTA ^{3'}	^{5'} -ACA ^{3'}	^{5'} -ACA ^{3'}	^{5'} -CCg ^{3'}			
				363			
				^{5'} -AgC ^{3'}			
				364			
				^{5'} -ggT ^{3'}			
				373			
				^{5'} -gCg ^{3'}			
3'-primer(s) ³	538	203	202	153	289	369	201
	5' -CCA 3'	^{5'} -CTg ^{3'}	5' -TCC 3'	5' -ACT 3'	^{5'} -AgC ^{3'}	5' -CCT 3'	5' -CTT 3'
		580	578	538	289	558	
		5' -TCC 3'	^{5'} -TgA ^{3'}	5' -CCA 3'	^{5'} -AgC ^{3'}	5' -Agg 3'	
		595	595				
		5' -CCT 3'	5' -CCT 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.

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Lot No.: 99V Lot-specific information

Lot No.: 99V Lot-specific information CELL LINE VALIDATION SHEET																				
HLA-C*02 SSP primer set ²																				
			116	.A-C 02		<u> </u>	М	1111	ICI	36	: L	W	الم							
					1	2	3	4	5	6	7	8	9	10	11	12	12	11	15	16
							3	4	3	0		0	9	10		12	13	14	13	10
				.:	9	302	903	70	305	90,	307	201071908	60	910	7	12	201437713	201184414	715	916
				ž	718	718	718	37.	718	37.	718	718	718	718	718	718	37.	847	37.	718
				Prod. No.	201071901	201071902	201071903	201437704	201071905	201437706	201071907	010	201071909	201071910	201071911	201071912	14	11	20143771	20107191
		10 11 11 1			7	Ñ	Ñ	Ñ	Ñ	Ñ	Ñ	Ñ	Ñ	Ñ	Ñ	Ñ	Ñ	Ñ	Ñ	Ñ
1 9001 SA		*07:02	<u>C*</u>	-	-	_	_	_	-	_	-	 	-	_	_	_	-	-	_	
2		LK707	*07:01	*15:05		-	H	-	-	-	-	-	-	-	-	-		-	-	+
3		E4181324	*12:02	10.00	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
4		GU373	*03:04	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*06:02		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*03:04	*07:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020		*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025		*04:01		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026		*12:03		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
10	9107		*01:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11 12	9051	PITOUT	*16:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13		JESTHOM	*06:02 *01:02		+	H	H	-	-	÷						-	-			-
14		OLGA	*01:02	*03:04	-	-	H	-	Ē		-	-	-	-	-	-		-	-	
15	9075		*03:04	00.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16		SWEIG007	*02:02		+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-
17		CTM3953540	*03:03	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*01:02	*07:05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20		SLE005	*03:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21		AMALA	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22		KOSE	*12:03	***	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
23	9124		*01:02	*15:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
24 25	9035	JBUSH	*12:03 *08:02		+	-	÷	-	-	-	-	-	-	-	-	-	-	-	-	+
26		WT49	*07:01		-	H	H	-	Ė	-	-	-	-	-	-	-	-	-	-	-
27		CH1007	*07:04	*15:05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
28		BEL5GB	*05:01	*16:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050		*16:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*17:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
31		DUCAF	*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297		*17:01	*17:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
33		MT14B	*03:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104		*12:03		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
35	9302	SSTO KT17	*05:01	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36 37		HHKB	*03:03 *07:02	*04:01	-	-	-	-	-	-	-	-	Ε.	-		-	÷	-	-	
38	9099		*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315		*02:02	*07:01	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-
40		WHONP199	*01:02	*06:02	+	÷	-	-	-	-	-	-	-	÷	-	-	-	-	-	-
41		H0301	*08:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*01:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*01:02	*08:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
44	9057		*12:03		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
45		SHJO	*06:02	*17:01	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
46		SCHU	*07:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47		TUBO	*07:04	*15:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
48	9303	TER-ND	*04:01	*16:01	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-



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Lot No.: 99V Lot-specific information

Lot No.: 99V Lot-specific information CELL LINE VALIDATION SHEET																			
HLA-C*02 SSP primer set ²																			
			HLA	\-C*02	SS	Ρį	ori	me	r s	eť									
			4-	W 7 18 19 20 21 22 23 2							_	00		00			0.4		
					17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
				Prod. No.:	201184417	201201618	201201619	201437720	201437721	201437722	201437723	201071924	201437725	201437726	201437727	201437728	201184429	201437730	201184431
	IHW	/C cell line ¹		<u>ш</u> С*	(1	(1	(1	(1	(1	(1	(1	(1	(1	(1	(1	(1	(1	(1	(1
1	9001		*07:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2		LK707	*07:01	*15:05	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3		E4181324	*12:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4		GU373	*03:04	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5		KAS011	*06:02		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353		*03:04	*07:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*05:01		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9007		*04:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*01:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*16:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052		*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		32367	*01:02	*07:05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19		BM16	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20		SLE005	*03:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21		AMALA	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22		KOSE	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124		*01:02	*15:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24		JBUSH	*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049		*08:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26		WT49	*07:01		-	•	•	-	-	-	-	•	-	-	•	•	•	•	-
27		CH1007	*07:04	*15:05	-	+	-	-	-	-	-	-	-	-	-	•	-	-	-
28		BEL5GB	*05:01	*16:01	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050		*16:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021		*17:01		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
31		DUCAF	*05:01	*47:00	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297		*17:01	*17:03	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
33		MT14B	*03:04		-	-	-	-	-	-	-	-	<u> </u>	-	-	-	-	-	-
34	9104		*12:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35		SSTO KT17	*05:01	*04.04	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
36		KT17	*03:03	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37		HHKB	*07:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38 39	9099 9315		*03:03 *02:02	*07:01	-	-	-	-	-	-	-	-	-	-	-		-	-	-
		WHONP199	*01:02	*06:02	-		-		-	-	-	-	-	-	-		-	-	
40 41		H0301	*08:02	00.02	-	+	-	-	-	-	-	÷	-	-	-	-	÷	-	ᆸ
41		TAB089	*01:02		-	-	-	-	-	-	-	-	Ë	-	-		-	-	-
42		T7526	*01:02	*08:01	-	-	-	-	-	-	-	-	-	-	-		-		
44	9076		*12:03	00.01	-	-	-	-	-	-	-	-	-	-	-		-	-	
44		SHJO	*06:02	*17:01	-		-	-	-	-	-	-	Ë	-	-		-	-	
46		SCHU	*07:02	17.01	-	+	-	÷		-	_	÷	Ē	-	-		-		
46		TUBO	*07:04	*15:02	-	<u>-</u>				-	-	÷	-		-				
48		TER-ND	*04:01	*16:01		+	-			-		÷	=	-				Ē	
40	9303	ו בע-ואט	04:01	10.01	-			-	-	-	-	<u> </u>	<u> </u>	•	-		-		-

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.

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Product Insert Page 15 of 16

101.622-12 - including *Taq* **pol.**, IFU-01 **101.622-12u - without** *Taq* **pol.**, IFU-02

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Lot No.: 99V Lot-specific information

²The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 3 to 8, 11, 12, 14, 15, 17, 19 to 23, 26, 27, 30 and 31 were available. The specificity of the primers in primer solutions 3 to 8, 11, 12 14, 15, 17, 19, 20, 22, 23, 26 30 and 31 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 21 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solutions 27 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 3, 8, 11, 13, 15, 17, 20, 23, 26, 28 and 30 one, two or three of the 3'-primers were not possible to test, and in primer solutions 4, 6, 10, 12, 15, 22, 23 and 28 one, two or three 5'-primers were not possible to test.

Additional primers in primer solutions 13, 25 and 28 were tested by separately adding either one 5'-primer, one or two 3'-primers.

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101.622-12 - including *Taq* **pol.**, IFU-01 **101.622-12u - without** *Taq* **pol.**, IFU-02

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Lot No.: 99V Lot-specific information

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