101.627-12 - including *Taq* polymerase, IFU-01
101.627-12u - without *Taq* polymerase, IFU-02

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

Lot No.: 90V

Lot-specific information

Olerup SSP® HLA-C*16

Product number:	**101.627-12 – including *Taq* polymerase** **101.627-12u – without *Taq* polymerase**
Lot number:	**90V**
Expiry date:	**2016-December-01**
Number of tests:	**12**
Number of wells per test:	**23+1**
Storage - pre-aliquoted primers:	**dark at -20ºC**
- PCR Master Mix:	**-20ºC**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 90V.

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*16 LOT (68S)

The HLA-C*16 kit is updated for new alleles to enable separation of:

- Confirmed[1] alleles as listed in the IMGT/HLA database
- Polymorphisms in exons outside of the region encoding the peptide binding domain
- Null and Alternatively expressed alleles

A well containing Negative Control primer pairs has been added.

The format of the Product Insert and Worksheet have been changed.

[1]As described in section Uniquely Identified Alleles.

The HLA-C*16 specificity and interpretation tables have been updated for the HLA-C alleles described since the previous *Olerup* SSP® HLA-C*16 lot was made **(Lot No. 68S)**.

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.

101.627-12 - including *Taq* polymerase, IFU-01
101.627-12u - without *Taq* polymerase, IFU-02

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Lot No.: 90V **Lot-specific information**

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

Well	5'-primer	3'-primer	rationale
6	Added	-	5'-primer added for the C*16:39:02 allele.
7	Added	-	5'-primer added for the C*16:45 allele.
8	Added	Added	Primer pair added for the C*16:68 allele.
12	-	Added	3'-primer added for the C*16:67 allele.
14	Added	-	5'-primer added from well 21.
15	Added	Modified	5'-primer added for the C*16:39:02 allele. Modified 3'-primer for improved HLA-specific amplification.
19	-	Added	3'-primer added for the C*16:67 allele.
20	Added	-	5'-primer added from well 21.
21	Moved, added	Added	5'-primer moved to wells 14 and 20, primer pair added from well 24, primer pair added for the C*16:63 allele.
22	Added	Added	Primer pair added for the C*16:58 alleles.
24	Moved	Moved	Primer pair moved to well 21, Negative Control.

Change in revision R01 compared to R00:

1. The C*16:68 allele is amplified by primer mix 1. This has been corrected in the Specificity and Interpretation Tables.

Lot No.: 90V **Lot-specific information**

Well **24** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup* SSP® HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

HLA-specific PCR product sizes range from 75 to 200 base pairs.
The PCR product generated by the positive control primer pair is 430 base pairs.

Length of PCR product	**105**	**200**	**105**	**80**	**75**	**80**	**85**
5'-primer[1]	**164**	**340**	**440**	**45**	**45**	**43**	**36**
	5'-CAC3'	**5'-Agg3'**	**5'-TTA3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-Tgg3'**	**5'-TAC3'**
							36
							5'-TAT3'
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**	**47**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**	**5'-ACA3'**
							48
							5'-gCA3'
							48
							5'-gCC3'
							52
							5'-TgT3'
A*	+	+	+				
B*	+	+	+				
C*	+	+	+				
DRB1				+	+		
DRB3				+	+		
DRB5				+			
DQB1					+		
DPB1						+	
DQA1							+

[1]The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and codonnumbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

[2]The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

101.627-12 - including *Taq* polymerase, IFU-01
101.627-12u - without *Taq* polymerase, IFU-02

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Lot No.: 90V **Lot-specific information**

PRODUCT DESCRIPTION

HLA-C*16 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the C*16:01 to C*16:70 alleles.

PLATE LAYOUT

Each HLA-C*16 test consists of 24 PCR reactions in a 24 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**	**NC**

The 24 well PCR plate is marked with 'HLA-C*16' in silver/gray ink.
Well No. 1 is marked with the Lot No. '90V'.
Wells 1 to 23 – HLA-C*16 high resolution primers.
Well 24 – Negative Control (NC).
A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.
The PCR plates are heat-sealed with a PCR-compatible foil.
Please note: When removing each 24 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Due to the sharing of sequence motifs between HLA-C alleles, non-HLA-C*16 alleles will be amplified by primer mixes 1 to 13, 15 to 19, and 22 to 23. In addition, a few HLA-A and HLA-B alleles will be amplified by primer mixes 4 to 7, 11 and 19.
For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-C*16 alleles, i.e. **C*16:01 to C*16:70**, recognized by the HLA Nomenclature Committee in April 2014[1,2] will be amplified by the primers in the HLA-C*16 SSP kit.

The HLA-C*16 kit enables separation of the confirmed HLA-C*16 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*16 alleles is listed below.

Lot No.: 90V **Lot-specific information**

The HLA-C*16 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*16 subtyping kit cannot distinguish the following silent mutations: the C*16:01:01, 16:01:03-16:01:15 and 16:01:17-16:01:18 alleles, the C*16:01:02 and 16:01:16 alleles, the C*16:02:01-16:02:12 alleles, the C*16:15:01-16:15:02 alleles or the C*16:39:01 and 16:39:02 alleles.

The following HLA-C*16 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
C*16:15:01-16:15:02, C*16:20	11	C*16:27, C*16:32	20
C*16:16Q, C*16:17	12	C*16:28, C*16:31, C*16:50	19
C*16:24, C*16:58	22	C*16:30N, C*16:56	23

[1]HLA-C alleles listed on the IMGT/HLA web page 2014-April-14, 3.16.0, www.ebi.ac.uk/imgt/hla.
[2]Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page http://hla.alleles.org/alleles/deleted.html.

101.627-12 - including *Taq* polymerase, IFU-01
101.627-12u - without *Taq* polymerase, IFU-02

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Lot No.: 90V **Lot-specific information**

ALLELE CONFIRMATION STATUS

Allele	Status[1]	Allele	Status[1]	Allele	Status[1]	Allele	Status[1]
C*16:01:01	**Confirmed**	**C*16:04:01**	**Confirmed**	C*16:33	Unconfirmed	C*16:62	Unconfirmed
C*16:01:02	Unconfirmed	C*16:06	Unconfirmed	C*16:34	Unconfirmed	C*16:63	Unconfirmed
C*16:01:03	**Confirmed**	C*16:07:01	Unconfirmed	**C*16:35**	**Confirmed**	C*16:64	Unconfirmed
C*16:01:04	**Confirmed**	**C*16:07:02**	**Confirmed**	C*16:36	Unconfirmed	C*16:65	Unconfirmed
C*16:01:05	**Confirmed**	**C*16:08**	**Confirmed**	C*16:37	Unconfirmed	C*16:66	Unconfirmed
C*16:01:06	Unconfirmed	C*16:09	Unconfirmed	**C*16:38**	**Confirmed**	**C*16:67**	**Confirmed**
C*16:01:07	Unconfirmed	C*16:10	Unconfirmed	**C*16:39:01**	**Confirmed**	C*16:68	Unconfirmed
C*16:01:08	Unconfirmed	C*16:11	Unconfirmed	C*16:39:02	Unconfirmed	C*16:69	Unconfirmed
C*16:01:09	**Confirmed**	C*16:12	Unconfirmed	**C*16:40**	**Confirmed**	C*16:70	Unconfirmed
C*16:01:10	Unconfirmed	**C*16:13**	**Confirmed**	C*16:41	Unconfirmed		
C*16:01:11	Unconfirmed	**C*16:14**	**Confirmed**	**C*16:42**	**Confirmed**		
C*16:01:12	Unconfirmed	**C*16:15:01**	**Confirmed**	C*16:43	Unconfirmed		
C*16:01:13	Unconfirmed	C*16:15:02	Unconfirmed	C*16:44	Unconfirmed		
C*16:01:14	Unconfirmed	**C*16:16Q**	**Confirmed**	**C*16:45**	**Confirmed**		
C*16:01:15	Unconfirmed	C*16:17	Unconfirmed	**C*16:46**	**Confirmed**		
C*16:01:16	Unconfirmed	**C*16:18**	**Confirmed**	C*16:47	Unconfirmed		
C*16:01:17	Unconfirmed	**C*16:19**	**Confirmed**	C*16:48	Unconfirmed		
C*16:01:18	Unconfirmed	C*16:20	Unconfirmed	**C*16:49**	**Confirmed**		
C*16:02:01	**Confirmed**	C*16:21	Unconfirmed	**C*16:50**	**Confirmed**		
C*16:02:02	**Confirmed**	**C*16:22**	**Confirmed**	C*16:51	Unconfirmed		
C*16:02:03	**Confirmed**	C*16:23	Unconfirmed	**C*16:52**	**Confirmed**		
C*16:02:04	Unconfirmed	**C*16:24**	**Confirmed**	C*16:53	Unconfirmed		
C*16:02:05	Unconfirmed	**C*16:25**	**Confirmed**	C*16:54	Unconfirmed		
C*16:02:06	Unconfirmed	**C*16:26**	**Confirmed**	C*16:55	Unconfirmed		
C*16:02:07	Unconfirmed	**C*16:27**	**Confirmed**	C*16:56	Unconfirmed		
C*16:02:08	Unconfirmed	C*16:28	Unconfirmed	C*16:57	Unconfirmed		
C*16:02:09	Unconfirmed	C*16:29	Unconfirmed	C*16:58	Unconfirmed		
C*16:02:10	Unconfirmed	**C*16:30N**	**Confirmed**	C*16:59	Unconfirmed		
C*16:02:11	Unconfirmed	C*16:31	Unconfirmed	C*16:60	Unconfirmed		
C*16:02:12	Unconfirmed	C*16:32	Unconfirmed	C*16:61	Unconfirmed		

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

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-C*16 homo- and heterozygotes is available upon request.

101.627-12 - including *Taq* polymerase, IFU-01
101.627-12u - without *Taq* polymerase, IFU-02

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Lot No.: 90V **Lot-specific information**

SPECIFICITY TABLE

HLA-C*16 SSP subtyping

Specificities and sizes of the PCR products of the 23+1 primer mixes used for HLA-C*16 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified HLA-C*16 alleles[3]	Other amplified HLA Class I alleles[4]
1	210 bp	**800 bp**	*16:01:01-16:02:12, 16:04:01, 16:08-16:21, 16:23-16:34, 16:36-16:39:02, 16:41-16:42, 16:44-16:47, 16:49-16:52, 16:54-16:70	*06:31
2[5,6]	75 bp	1070 bp	*16:02:01-16:02:12, 16:09, 16:12, 16:19, 16:25, 16:46-16:48, 16:57, 16:60, 16:63, 16:69	*01:14, 01:59, 02:02:01-02:02:03, 02:02:05-02:02:11, 02:02:13-02:11, 02:13-02:26:03, 02:28-02:40, 02:42-02:65, 02:67Q-02:81, 03:07, 03:15, 03:45, 03:130, 03:140, 03:163, 03:243, 04:01:01:01-04:01:28, 04:01:30-04:01:59, 04:03:01-04:10, 04:12-04:20, 04:23-04:28, 04:30-04:35, 04:37-04:54, 04:56-04:91, 04:93N-04:111, 04:113-04:168, 04:170N, 05:01:01:01-05:01:27, 05:03-05:35, 05:37-05:102, 06:02:01:01-06:02:01:03, 06:02:03-06:02:11, 06:02:14-06:10, 06:12-06:43:02, 06:45-06:51, 06:53:01-06:121, 06:123, 06:125-06:131, 07:49, 07:76:01-07:76:02, 07:238, 07:315, 07:328, 08:10, 12:04:01-12:05, 12:09, 12:21, 12:33, 12:41, 12:54, 12:60, 14:04, 14:12, 14:49, 14:64, 15:11, 15:16-15:17, 17:01:01:01-17:21, 17:23-17:24, 18:01-18:04, 18:06-18:07N
3[6]	220 bp	**800 bp**	*16:04:01, 16:29, 16:33, 16:42, 16:55, 16:61, 16:66	*01:04, 01:09, 02:05:01-02:05:03, 02:17, 06:02:01:01-06:02:01:03, 06:02:03-06:02:15, 06:02:17-06:03:02, 06:07-06:13, 06:15-06:34, 06:36-06:39, 06:41-06:71, 06:73-06:78, 06:80, 06:82-06:100, 06:102-06:117, 06:119-06:122, 06:124-06:126, 06:128N-06:131, 12:03:01:01-12:07, 12:11-12:13, 12:15, 12:23, 12:25-12:26, 12:28-12:29, 12:31-12:35, 12:37-12:39N, 12:42Q-12:43, 12:45-12:48, 12:50-12:55, 12:57-12:63, 12:65-12:66, 12:70-12:71, 12:75-12:79, 12:81-12:82, 12:87-12:95, 12:97-12:102, 12:107-12:111, 12:113, 12:115-12:116, 12:119-12:122, 12:125, 14:16
4[7]	140 bp	**800 bp**	*16:01:01, 16:01:03-16:01:15,16:01:17-16:01:18, 16:04:01, 16:06-	*01:21, 02:12[w], 02:27:01-02:27:02, 03:04:25, 04:11, 04:29, 04:36, 04:55, 07:02:09, 08:01:01-08:02:10, 08:03:01-08:09, 08:11-08:63, 08:65-08:94, 08:95[w], 08:96-08:106, 12:02:01-12:02:10, 12:03:01:01-12:03:03, 12:03:05-12:03:08,

CE

			16:08, 16:10-16:11, 16:13-16:18, 16:20-16:24, 16:26-16:36, 16:37[w], 16:38-16:45, 16:49-16:56, 16:58-16:59, 16:61-16:62, 16:64-16:68	12:03:10-12:03:23, 12:03:24[w], 12:03:25-12:03:30, 12:06-12:08, 12:10:01-12:20, 12:22-12:26, 12:28-12:32, 12:34-12:40, 12:42Q-12:53, 12:55-12:59, 12:61-12:71, 12:72[w], 12:73-12:122, 12:124-12:125, 14:02:03, 14:03, 14:08, 14:10, 14:22, 14:35N, 14:38, 14:41, 14:53-14:54, 14:61, 15:07, 15:21[w], 15:25, **B*35:08:02, B*35:08:05, B*67:02**
5	160 bp	**800 bp**	*16:01:01-16:02:12, 16:06-16:09, 16:11-16:28, 16:30N-16:32, 16:34, 16:36-16:39:02, 16:41, 16:43-16:44, 16:46-16:47, 16:49-16:52, 16:54, 16:56-16:60, 16:62-16:65, 16:69-16:70	*07:53, 07:216, **A*24:174**, **B*46:25**
6[5]	125 bp	**800 bp**	*16:11, 16:39:01-16:39:02	*02:21
	160 bp		*16:10	**A*24:106**
	210 bp		*16:06	*07:216
7[5]	100 bp	1070 bp	*16:09	*02:34
	170 bp		*16:45	*04:14, 04:68, **A*24:96, A*24:146**
	210 bp		*16:07:01	**B*46:25**
8	130 bp	1070 bp	*16:08, 16:38	*08:96
	435 bp		*16:53, 16:68	
9[5,7]	85 bp	1070 bp	*16:12	*02:79, 04:69
	140 bp		*16:52	
	205 bp		*16:35, 16:48	*06:118, 07:31:01-07:31:02, 07:177, 14:15
10	215 bp	**800 bp**	*16:19	*04:101, 07:114
	350 bp		*16:13, 16:61	*05:81, 06:87, 07:24, 07:218, 12:45, 14:65
11[7]	170 bp	1070 bp	*16:20	15:75, **A*24:73, A*24:157, B*07:66, B*46:25, B*51:55**
	540 bp		*16:15:01-16:15:02, 16:25, 16:64	*04:14, 04:68, 07:53, 07:216
12[5]	100 bp	1070 bp	*16:17, 16:67	*01:27
	210 bp		*16:22	
	245 bp		*16:16Q	
13	130 bp	1070 bp	*16:14	*06:32, 12:40
14[7]	210 bp	1070 bp	*16:18, 16:23	
15[5]	120 bp	1070 bp	*16:39:01-16:39:02	
	145 bp		*16:21	*02:14, 04:42, 05:43, 06:05, 07:02:09, 08:37, 12:16, 15:23, 15:63

16	375 bp	1070 bp	*16:06-16:07:02	*01:05, 01:21, 01:36, 01:55, 01:79:01-01:79:02, 02:02:01-02:02:03, 02:02:05-02:02:08, 02:02:10-02:04, 02:06-02:16:02, 02:18-02:36, 02:38N-02:40, 02:42-02:56, 02:58-02:61, 02:63-02:73, 02:75-02:80, 03:05, 03:13:01-03:13:02, 03:25, 03:27, 03:35, 03:135, 03:167, 03:178, 03:198, 04:01:01:01-04:01:23, 04:01:25-04:01:59, 04:03:01-04:20, 04:23-04:36, 04:38-04:39, 04:41-04:79, 04:81-04:99, 04:101-04:109, 04:111-04:116, 04:118-04:170N, 05:01:01:01-05:01:20, 05:01:22-05:01:27, 05:03-05:06, 05:08-05:09:02, 05:11-05:15, 05:17-05:30, 05:32-05:84, 05:86-05:95, 05:97-05:102, 06:101, 06:127, 07:01:01:01-07:01:10, 07:01:12-07:01:27, 07:01:29-07:03, 07:05-07:09, 07:13-07:30, 07:32N-07:33N, 07:35-07:42, 07:44, 07:46-07:62, 07:64-07:100, 07:102-07:138, 07:140-07:141:02, 07:143-07:176, 07:178-07:180, 07:182-07:183, 07:185-07:194, 07:197-07:271, 07:273-07:294, 07:296-07:301, 07:303-07:322, 07:325-07:327, 07:330-07:331, 07:333-07:335, 07:337, 07:339-07:345, 07:347N-07:353, 07:356, 07:359-07:360, 07:362-07:363, 07:368-07:374, 08:01:01-08:01:10, 08:01:12-08:11, 08:13-08:33:03, 08:35-08:43, 08:45-08:60, 08:62-08:63, 08:65-08:81, 08:83-08:106, 12:02:01-12:02:11, 12:08, 12:10:01-12:10:02, 12:14:01-12:14:02, 12:16-12:18:02, 12:21-12:22, 12:27, 12:30, 12:36, 12:40-12:41, 12:49, 12:56, 12:64, 12:67-12:69, 12:72-12:74, 12:80N, 12:83-12:86, 12:96, 12:103-12:106, 12:112, 12:114, 12:117-12:118, 12:123-12:124, 14:09, 15:22, 15:65, 15:72, 17:16, 18:01-18:07N
17	180 bp	1070 bp	*16:26, 16:46, 16:55, 16:64	*02:49, 02:75, 04:01:01:01-04:01:09, 04:01:11-04:01:22, 04:01:24-04:01:59, 04:03:01-04:10, 04:12-04:20, 04:23-04:26, 04:28-04:32, 04:34-04:51, 04:53-04:54, 04:56-04:106, 04:108-04:115N, 04:117-04:129, 04:131-04:168, 04:170N, 05:25, 05:42, 06:05, 06:76:02, 07:02:09, 08:28, 12:28, 15:25, 15:62
18[5]	120 bp 255 bp	1070 bp	*16:38 *16:26, 16:46, 16:55, 16:64	*08:96 *01:23, 01:58, 02:49, 02:75, 04:03:01-04:03:02, 04:06, 04:80, 04:140, 04:147, 04:160, 05:25, 05:42, 06:02:01:01-06:02:01:03, 06:02:03-06:02:09, 06:02:11-06:25, 06:27-06:29, 06:31-06:52, 06:54-06:124, 06:126-06:131, 07:01:01:01-07:01:22, 07:01:24-07:02:10, 07:02:12-07:02:59, 07:04:01-07:04:04, 07:04:06-07:06, 07:08-07:15, 07:17:01-07:19, 07:21-07:33N, 07:35, 07:37-07:50, 07:52-07:55N, 07:57-07:58, 07:61N-07:63, 07:65-07:78, 07:80-07:87, 07:89-07:95, 07:96:02-07:108:02, 07:110-07:126, 07:128-07:172:01, 07:173-07:176, 07:178-07:180, 07:182-07:226, 07:228-07:262, 07:264N-07:294, 07:296-07:326,

Lot No.: 90V **Lot-specific information**

				07:329N-07:354, 07:356-07:366, 07:368-07:374, 08:28, 12:28, 15:25, 15:62, 17:11, 18:01-18:07N
19[5]	105 bp 245 bp	**800 bp**	*16:28, 16:67 *16:29, 16:31, 16:50	*06:90 *01:10, 02:05:01-02:05:03, 02:17, 06:08, 06:22 12:119, 14:25, 17:21, **B*40:243**
20[5]	95 bp 145 bp 210 bp	1070 bp	*16:27 *16:32 *16:23	
21	215 bp 445 bp 595 bp	1070 bp	*16:63 *16:40, 16:53 *16:49	
22[5]	85 bp 210 bp	**800 bp**	*16:58 *16:24	 *03:108, 03:150, 07:25
23[5,7]	95 bp 170 bp	1070 bp	*16:42, 16:56 *16:30N	*05:56, 08:69
24[8]	-	-	**Negative Control**	

[1]Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of HLA-C*16 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.

[2]The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

[3]For several HLA Class I alleles 1st and/or 4th exon(s) and beyond, as well as intron nucleotide sequences, are not available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

[4]Due to the sharing of sequence motifs between HLA-C alleles, non-HLA-C*16 alleles will be amplified by primer mixes 1 to 13, 15 to 19, and 22 to 23. In addition, a few HLA-A and HLA-B alleles will be amplified by primer mixes 4 to 7, 11 and 19.

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

[6]Primer mixes 2 and 3 may give rise to a lower yield of HLA-specific PCR product than the other C*16 primer mixes.

[7]Primer mixes 4, 9, 11, 14 and 23 may have tendencies of unspecific amplifications, most pronounced in primer mix 14.

[8]Primer mix 24 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.

101.627-12 - including *Taq* polymerase, IFU-01
101.627-12u - without *Taq* polymerase, IFU-02

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Lot No.: 90V **Lot-specific information**

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	210	75	220	140	160	125	100	130	85	215	170	100
PCR product						160	170	435	140	350	540	210
						210	210		205			245
Length of int. pos. control[1]	800	1070	800	800	800	800	1070	1070	1070	800	1070	1070
5'-primer(s)[2]	360	270	361	201	419	113	244	126	256	385	289	361
	5' -CAg 3'	5' -AAg 3'	5' -AgT 3'	5' -CCA 3'	5' -gTC 3'	5' -CCA 3'	5' -CgC 3'	5' -ggA 3'	5' -ACg 3'	5' -ggT 3'	5' -Agg 3'	5' -AgT 3'
	361					124	369	539	361	523	409	
	5' -AgT 3'					5' -gCC 3'	5' -TAC 3'	5' -gCg 3'	5' -AgT 3'	5' -CCg 3'	5' -ggC 3'	
						124	412					
						5' -gCA 3'	5' -ATA 3'					
						368						
						5' -gTC 3'						
						418						
						5' -Agg 3'						
3'-primer(s)[3]	527	302	538	302	539	201	302	205	302	3rd I	539	413
	5' -CCg 3'	5' -ggT 3'	5' -CCA 3'	5' -ggC 3'	5' -TCT 3'	5' -CTT 3'	5' -ggT 3'	5' -CCT 3'	5' -ggT 3'	5' -CTC 3'	5' -TCT 3'	5' -gCC 3'
	527					539	539	220	461			427
	5' -CCg 3'					5' -TCT 3'	5' -TCT 3'	5' -CgA 3'	5' -gCT 3'			5' -gTA 3'
								3rd I	527			530
								5' -gCA 3'	5' -CCg 3'			5' -CCA 3'
												563
												5' -CgT 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
Length of spec.	130	210	120	375	180	120	105	95	215	85	95
PCR product			145			255	245	145	445	210	170
								210	595		
Length of int. pos. control[1]	1070	1070	1070	1070	1070	1070	800	1070	1070	800	1070
5'-primer(s)[2]	126	368	97	361	201	126	361	368	379	524	201
	5' -ggA 3'	5' -gTg 3'	5' -TCg 3'	5' -AgA 3'	5' -CCA 3'	5' -ggA 3'	5' -AgT 3'	5' -gTT 3'	5' -ACg 3'	5' -CCA 3'	5' -CCA 3'
		368	124					436	527	3rd I	
		5' -gTT 3'	5' -gCC 3'					5' -AgA 3'	5' -TgA 3'	5' -Cgg 3'	
			124					485	1019		
			5' -gCA 3'					5' -CAA 3'	5' -TgA 3'		
3'-primer(s)[3]	214	539	201	3rd I	341	205	413	539	3rd I	3rd I	257
	5' -CCA 3'	5' -TCT 3'	5' -CTT 3'	5' -CTC 3'	5' -CgT 3'	5' -CCT 3'	5' -gCC 3'	5' -TCT 3'	5' -gCA 3'	5' -CTC 3'	5' -CCT 3'
						341	430		1087	632	331
						5' -CgT 3'	5' -gCg 3'		5' -AgC 3'	5' -gTA 3'	5' -CTA 3'
							559				
							5' -CTC 3'				
							565				
							5' -CAT 3'				
Well No.	13	14	15	16	17	18	19	20	21	22	23

[1]The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 bp, internal positive control band.

101.627-12 - including *Taq* polymerase, IFU-01
101.627-12u - without *Taq* polymerase, IFU-02

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Lot No.: 90V **Lot-specific information**

The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

[2]The nucleotide position matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

[3]The nucleotide position matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

101.627-12 - including *Taq* polymerase, IFU-01
101.627-12u - without *Taq* polymerase, IFU-02

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Lot No.: 90V **Lot-specific information**

CELL LINE VALIDATION SHEET

HLA-C*16 SSP primer set[2]

					Well														
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.:	201186001	201206402	201073703	201206404	201073705	201436906	201436907	201436908	201206409	201073710	201073711	201436912	201073713	201436914	201436915	201186016
	IHWC 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	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+

101.627-12 - including *Taq* polymerase, IFU-01
101.627-12u - without *Taq* polymerase, IFU-02

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Lot No.: 90V **Lot-specific information**

CELL LINE VALIDATION SHEET

HLA-C*16 SSP primer set[2]

					Well						
					17	18	19	20	21	22	23
				Prod. No.:	201436917	201206418	201436919	201436920	201436921	201436922	201206423
	IHWC 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	+	-	-	-	-	-	-

101.627-12 - including *Taq* polymerase, IFU-01 Visit www.olerup-ssp.com for
101.627-12u - without *Taq* polymerase, IFU-02 **"Instructions for Use" (IFU)**

Lot No.: 90V **Lot-specific information**

[1]The provided cell line HLA specificities are retrieved from the http://www.ihwg.org/hla web site. The specificity of an individual cell line may thus be subject to change.

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

No DNAs carrying the alleles to be amplified by primer solutions 6 to 15 and 19 to 23 were available. The specificity of the primers in primer solutions 6 to 9, 11, 12, 14, 15 and 19 to 22 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 10 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solutions 13 and 23 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 1, 6, 7, 15, 20 and 21 one to four of the 5'-primers were not possible to test, and in primer solutions 8, 9, 12, 18, 19 and 22 one to three of the 3'-primers were not possible to test.

101.627-12 - including *Taq* polymerase, IFU-01
101.627-12u - without *Taq* polymerase, IFU-02

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Lot No.: 90V **Lot-specific information**

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