

101.421-06 – including *Taq* polymerase, IFU-01
101.421-06u – without *Taq* polymerase, IFU-02

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“Instructions for Use” (IFU)

Lot No.: **59V**

Lot-specific Information
Olerup SSP® HLA-A*23

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Product number:	101.421-06 – including <i>Taq</i> polymerase 101.421-06u – without <i>Taq</i> polymerase
Lot number:	59V
Expiry date:	2016-October-01
Number of tests:	6
Number of wells per test:	31+1
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

This Product Description is only valid for Lot No. 59V.

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-A*23 LOT (68R)

The HLA-A*23 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.

¹As described in section Uniquely Identified Alleles.

The HLA-A*23 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP®* HLA-A*23 lot was made (Lot No. 68R).

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

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

Well	5'-primer	3'-primer	rationale
4	-	Added	3'-primer added for the A*23:58 allele.
5	Modified	-	5'-primer modified for improved specificity.
7	-	Added	3'-primer added from well 26.
8	-	Added	3'-primer added from well 26.
11	Added	-	5'-primer added from well 28.
12	Modified	Modified	5'-primer and 3'-primer modified for improved specificity.
19	Added	-	5'-primer added from well 32.
20	Added	-	5'-primer added from well 32, 5'-primer added from well 28.
23	-	Added	3'-primer added for the A*23:58 allele.
26	Exchanged	Modified, moved	5'-primer exchanged and 3'-primer modified for the A*23:36 allele, 3'-primers moved to wells 7, 8 and 29.
27	-	Added	3'-primer added for the A*23:57 allele.
28	Added, moved	Added, moved	Primer pair added for the A*23:17 allele, 5'-primers moved to wells 11 and 20.
29	-	Added	3'-primer added from well 26.
30	-	Added	3'-primer added for the A*23:56 allele.
32	Moved	Moved	Primer pairs moved to wells 19 and 20, Negative control.

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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 and DPB1 amplicons as well as amplicons generated by a control primer pair.

PCR product sizes range from 75 to 430 base pairs.
The PCR product generated by the control primer pair is 430 base pairs.

Length of PCR product	105	200	105	80	75	80
5'-primer¹	164	340	440	45	45	43
	5'-CAC ^{3'}	5'-Agg ^{3'}	5'-TTA ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}
3'-primer²	231	2nd I	507	59	58	57
	5'-TgC ^{3'}	5'-AAA ^{3'}	5'-TTg ^{3'}	5'-CTC ^{3'}	5'-ggC ^{3'}	5'-CTC ^{3'}
A*	+	+	+			
B*	+	+	+			
C*	+	+	+			
DRB1				+	+	
DRB3				+	+	
DRB5				+		
DQB1					+	
DPB1						+

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-A*23 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*23:01 to A*23:60 alleles.

PLATE LAYOUT

Each 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 cut PCR plate is marked with 'HLA-A*23' in silver/gray ink.

Well No. 1 is marked with the Lot Number '59V'.

Wells 1 to 31 – HLA-A*23 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 covered with a PCR-compatible foil.

Please note: When removing each 32 well PCR plate, make sure that the remaining plates stay covered. 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-A alleles non-HLA-A*23 alleles will be amplified by primer mixes 1 to 10, 12 to 20 and 22 to 30. In addition, the B*18:27 alleles will be amplified by primer mixes 1, 3, 10, 14 and 15. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*23 alleles, i.e. **A*23:01 to A*23:60 alleles**, recognized by the HLA Nomenclature Committee in January 2014^{1,2} will be amplified by the primers in the HLA-A*23 subtyping kit.

The HLA-A*23 kit enables separation of the confirmed HLA-A*23 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-A*23 alleles is listed below.

The HLA-A*23 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles

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The HLA-A*23 subtyping kit cannot distinguish the silent mutations in the A*23:01:01-23:01:13 alleles, the A*23:03:01-23:03:02 alleles and the A*23:37:01-23:37:02 alleles.

The following HLA-A*23 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
A*23:08N, 23:22	7	A*23:16, 23:29	20
A*23:09, 23:26	8	A*23:18, 23:28	4
A*23:10, 23:23	10	A*23:19Q, 23:31, 23:53	22
A*23:13, 23:33	17	A*23:41, 23:42	25
A*23:15, 23:27	19		

¹HLA-A alleles listed on the IMGT/HLA web page 2014-January-17, release 3.15.0, www.ebi.ac.uk/imgt/hla.

²Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page <http://hla.alleles.org/alleles/deleted.html>.

ALLELE CONFIRMATION STATUS

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

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2014-January-17, release 3.15.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-A*23 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

HLA-A*23 SSP subtyping

Specificities and sizes of the PCR products of the 31+1 primer mixes used for HLA-A*23 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*23 alleles ³	Other amplified HLA Class I alleles ⁴
1 ⁶	210 bp	800 bp	*23:01:01-23:01:13, 23:03:01-23:60	*02:17:01-02:17:03, 02:108, 02:110, 02:268, 02:300, 02:303, 24:13:01-24:13:02, 24:18, 24:24, 24:94, 24:188, 24:207, 24:228, 29:07, 29:49, 31:29, B*18:27
2	160 bp	1070 bp	*23:01:01-23:02, 23:05- 23:42, 23:44-23:60	*02:19, 02:36-02:37, 02:54, 02:255, 02:417, 24:02:01:01-24:02:41, 24:02:43-24:02:69, 24:02:71-24:02:83, 24:04-24:09N, 24:11N, 24:13:01-24:15, 24:17, 24:19-24:20, 24:24- 24:32, 24:34-24:64, 24:66-24:74:02, 24:76- 24:93, 24:95-24:109, 24:111-24:124, 24:126-24:137, 24:139-24:157, 24:159- 24:166, 24:168-24:203, 24:205-24:206, 24:209, 24:212-24:218, 24:220-24:270, 33:19, 68:26, 68:65
3 ⁵	125 bp	800 bp	*23:03:01-23:03:02	*11:139, 24:21:01, 24:208, 29:07, 29:49, 31:29, B*18:27
	270 bp		*23:25	
4 ⁵	90 bp	1070 bp	*23:18	*24:267
	170 bp		*23:58	
	200 bp		*23:28	*24:77
5	230 bp	800 bp	*23:06	*31:36
6	470 bp	1070 bp	*23:07N	*01:04N, 03:21N, 11:21N, 24:11N
7 ⁵	95 bp	800 bp	*23:08N	*02:82N
	145 bp		*23:39	*30:22
	205 bp		*23:22	
8 ⁷	140 bp	1070 bp	*23:26, 23:39	*30:22
	170 bp		*23:48	
	215 bp		*23:09	*01:02, 01:20, 24:129
9	235 bp	1070 bp	*23:05	*24:25
10	135 bp	800 bp	*23:23	B*18:27
	235 bp		*23:10, 23:43	*11:139 ^w , 24:10:01 ^w -24:10:02 ^w , 24:46, 24:210 ^w
11	200 bp	800 bp	*23:11N	
	270 bp		*23:32	
12	190 bp	1070 bp	*23:12	*24:30, 24:42, 25:11, 32:08
13 ⁵	90 bp	800 bp	*23:30	
	210 bp		*23:02, 23:24	*24:06, 24:87, 24:138, 24:167
14	245 bp	1070 bp	*23:04	*02:17:01-02:17:03, 02:108, 02:110, 02:268, 02:300, 02:303, 11:139, 24:03:01- 24:03:02, 24:10:01-24:10:02, 24:18, 24:22,

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				24:33, 24:94, 24:125, 24:138, 24:167, 24:204, 24:207, 24:210, 29:07, 29:49, 31:29, B*18:27
15	210 bp 290 bp	1070 bp	*23:14:01 *23:47	*24:13:02, B*18:27 *24:234
16	175 bp 205 bp	800 bp	*23:14:01-23:14:02	*02:17:01 ^W -02:17:03 ^W , 11:139, 24:02:01:01-24:04, 24:06-24:11N, 24:13:01-24:13:02, 24:17-24:23, 24:24, 24:25-24:50, 24:54-24:56, 24:58-24:63, 24:66-24:91, 24:93, 24:95-24:113, 24:115-24:129, 24:131-24:137, 24:139-24:167, 24:169-24:187, 24:189-24:193, 24:195-24:198, 24:200-24:210, 24:212-24:221, 24:223-24:227, 24:229-24:270, 33:19 *11:139, 24:02:01:01-24:02:15, 24:02:17-24:05, 24:07-24:11N, 24:17, 24:19-24:21:02, 24:23, 24:24, 24:25-24:50, 24:55-24:56, 24:58-24:63, 24:66-24:86N, 24:88-24:90N, 24:93, 24:95-24:106, 24:108-24:113, 24:115-24:132N, 24:134-24:137, 24:139-24:166, 24:168-24:187, 24:189-24:206, 24:208-24:210, 24:212-24:221, 24:223-24:226:02, 24:229-24:270, 26:16, 33:19, 68:45
17⁵	125 bp 225 bp	1070 bp	*23:33 *23:13	*03:72, 11:88, 24:07, 24:19, 24:24, 24:131, 29:37, 29:56, 30:01:01-30:01:09, 30:11:01-30:11:02, 30:14L-30:20, 30:23-30:26, 30:30-30:31, 30:35-30:43, 30:48-30:49, 30:52-30:54, 30:56, 30:58-30:60, 30:62-30:63, 30:65, 30:72-30:75, 30:78N, 68:45
18⁵	110 bp	1070 bp	*23:01:01-23:01:13, 23:02 ^W , 23:04-23:13, 23:14:01 ^W , 23:14:02-23:23, 23:25-23:33, 23:35-23:56, 23:58-23:60	*02:40:01, 02:40:02 ^W , 02:51, 02:130, 24:24, 31:67-31:68, 32:28, 33:32:01, 68:51 ^W
19⁵	115 bp 195 bp 260 bp	800 bp	*23:15, 23:49 *23:46 *23:27	*30:06 *24:66, 30:75 *24:255
20	130 bp 195 bp 230 bp 270 bp	800 bp	*23:29 *23:46 *23:16 *23:32	*24:128 *24:66, 30:75
21⁵	90 bp	800 bp	*23:01:01-23:01:13, 23:02 [?] -23:04 [?] , 23:06-23:07N, 23:08N [?] , 23:09, 23:10 [?] -23:16 [?] , 23:18 [?] -23:25 [?] , 23:26, 23:27 [?] -23:37:02 [?] , 23:38N, 23:39 [?] -23:60 [?]	
22^{5,6}	90 bp 290 bp	1070 bp	*23:31, 23:45, 23:53 *23:19Q	*11:139, 24:17, 24:41, 24:62, 24:106, 24:208, 29:07, 29:49, 31:29

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23^{5,7}	80 bp	1070 bp	*23:45	*02:41, 02:80, 02:117, 02:289, 02:304, 02:454, 24:62, 26:10, 31:67-31:68, 32:28, 33:32:01
	170 bp		*23:20, 23:58	
24⁵	105 bp	800 bp	*23:49	*30:06
	180 bp		*23:21	
	240 bp		*23:40	
25⁵	95 bp	800 bp	*23:41	*02:221, 29:15, 31:78
	205 bp		*23:42	
26⁵	80 bp	1070 bp	*23:36	*24:32, 32:05
27⁶	195 bp	1070 bp	*23:57	*02:17:01-02:17:03, 02:108, 02:110, 02:268, 02:300, 02:303, 24:13:01, 24:94, 24:188, 24:207, 24:228
	205 bp		*23:34	
	285 bp		*23:35	
28	385 bp	800 bp	*23:02 [?] -23:04 [?] , 23:05, 23:08N [?] , 23:10 [?] -23:16 [?] , 23:17, 23:18 [?] -23:25 [?] , 23:27 [?] -23:37:02 [?] , 23:39 [?] -23:60 [?]	*24:02:01:01-24:02:09, 24:02:11-24:04, 24:05 [?] -24:06 [?] , 24:07-24:10:01, 24:10:02 [?] , 24:11N, 24:13:01 [?] -24:15 [?] , 24:17 [?] -24:19 [?] , 24:20, 24:21:01 [?] -24:21:02 [?] , 24:22, 24:23 [?] -24:28 [?] , 24:29, 24:30 [?] -24:32 [?] , 24:34 [?] -24:43 [?] , 24:44, 24:45N [?] -24:60N [?] , 24:61, 24:62 [?] -24:64 [?] , 24:66 [?] -24:78 [?] , 24:79, 24:80 [?] -24:85 [?] , 24:86N, 24:87 [?] -24:94 [?] , 24:95, 24:96 [?] -24:127 [?] , 24:128-24:129, 24:130 [?] -24:131 [?] , 24:132N, 24:133 [?] -24:141 [?] , 24:142-24:143, 24:144 [?] -24:151 [?] , 24:152, 24:153 [?] -24:162 [?] , 24:163N, 24:164 [?] -24:193 [?] , 24:194, 24:195 [?] -24:210 [?] , 24:212 [?] -24:214 [?] , 24:215, 24:216 [?] -24:225:01 [?] , 24:225:02, 24:226:01 [?] , 24:226:02, 24:227 [?] -24:230 [?] , 24:231, 24:232N [?] , 24:233-24:235, 24:236 [?] -24:247 [?] , 24:248-24:251, 24:252N [?] -24:253 [?] , 24:254-24:256, 24:257 [?] -24:258 [?] , 24:259-24:270, 33:19 [?]
29	170 bp	1070 bp	*23:48	
	200 bp		*23:37:01-23:37:02	
	240 bp		*23:40	
	290 bp		*23:47	*24:234
30⁵	110 bp	1070 bp	*23:56	
	245 bp		*23:38N	*24:61
31	185 bp	1070 bp	*23:44	
32⁸	Negative Control			

¹Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of HLA-A*23 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.

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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-A alleles non-HLA-A*23 alleles will be amplified by primer mixes 1 to 10, 12 to 20 and 22 to 30. In addition, the B*18:27 alleles will be amplified by primer mixes 1, 3, 10, 14 and 15.

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

⁶Primer mixes 1, 22 and 27 may give rise to a lower yield of HLA-specific PCR product than the other A*23 primer mixes.

⁷Primer mix 8 and 23 may have tendencies of unspecific amplifications.

⁸Primer 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', may be weakly amplified.

'?', nucleotide sequence information not available for the primer matching sequence.

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	210	160	125	90	230	470	95	140	235	135	200	190
PCR product			270	170			145	170		235	270	
				200			205	215				
Length of int. pos. control ¹	800	1070	800	1070	800	1070	800	1070	1070	800	800	1070
5'-primer(s) ²	368	453	368	98	144	3 rd I	98	98	28	368	90	144
	5'-gTT 3'	5'-AAA 3'	5'-gTT 3'	5'-CTC 3'	5'-gCC 3'	5'-ATA 3'	5'-CTC 3'	5'-CTC 3'	5'-TCg 3'	5'-gTT 3'	5'-AgT 3'	5'-gCC 3'
				678			564				160	
				5'-AgA 3'			5'-TgA 3'				5'-ACg 3'	
3'-primer(s) ³	539	570	453	256	331	621	200	193	92	463	317	292
	5'-TCA 3'	5'-CCg 3'	5'-TCg 3'	5'-CTg 3'	5'-CTC 3'	5'-ggg 3'	5'-TCC 3'	5'-CgA 3'	5'-AAC 3'	5'-gCT 3'	5'-ggA 3'	5'-gTg 3'
			595	728			262	200		559		
			5'-CCg 3'	5'-CCT 3'			5'-TgC 3'	5'-TCC 3'		5'-CCg 3'		
				809			616	227		571		
				5'-CAA 3'			5'-CgT 3'	5'-CTg 3'		5'-CCT 3'		
								271				
								5'-CAT 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.	90	245	210	175	125	110	115	130	90	90	80	105
PCR product	210		290	205	225		195	195		290	170	180
							260	230				240
								270				
Length of int. pos. control ¹	800	1070	1070	800	1070	1070	800	800	800	1070	1070	800
5'-primer(s) ²	368	368	368	98	98	453	98	90	920	368	414	98
	5'-gTT 3'	5'-gTT 3'	5'-gTT 3'	5'-CTC 3'	5'-CTC 3'	5'-AAA 3'	5'-CTC 3'	5'-AgT 3'	5'-CCA 3'	5'-gTT 3'	5'-CAg 3'	5'-CTC 3'
				368			418	228			678	
				5'-gTT 3'			5'-AgC 3'	5'-ATg 3'			5'-AgA 3'	
							493	379				
							5'-CTg 3'	5'-ACA 3'				
								418				
								5'-AgC 3'				
3'-primer(s) ³	419	570	538	259	181	524	163	317	968	418	453	163
	5'-CgC 3'	5'-CAC 3'	5'-CAg 3'	5'-gTT 3'	5'-gTA 3'	5'-CAC 3'	5'-CgC 3'	5'-ggA 3'	5'-CAg 3'	5'-gTC 3'	5'-TCT 3'	5'-CgC 3'
			616	502	282		316	570		619	806	238
	5'-CCA 3'		5'-CgC 3'	5'-CTT 3'	5'-gAC 3'		5'-gAA 3'	5'-CCg 3'		5'-gTT 3'	5'-CTA 3'	5'-CCT 3'
				539	282		570				809	299
	5'-TCC 3'			5'-TCT 3'	5'-gAC 3'		5'-CCg 3'				5'-CAA 3'	5'-TCg 3'
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

101.421-06 – including *Taq* polymerase, IFU-01
101.421-06u – without *Taq* polymerase, IFU-02

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Well No.	25	26	27	28	29	30	31
Length of spec.	95	80	195	385	170	110	185
PCR product	205		205		200	245	
			285		240		
					290		
Length of int.	800	1070	1070	800	1070	1070	1070
pos. control ¹							
5'-primer(s) ²	376	265	368	678	98	98	395
	5'-gCT 3'	5'-CAg 3'	5'-gTT 3'	5'-AgA 3'	5'-CTC 3'	5'-CTC 3'	5'-gCA 3'
	484				368		
	5'-ACg 3'				5'-gTT 3'		
3'-primer(s) ³	538	302	524	920	227	167	538
	5'-CAA 3'	5'-ggC 3'	5'-CAT 3'	5'-Tgg 3'	5'-CTg 3'	5'-ACC 3'	5'-CAA 3'
			534		255	303	
			5'-CgT 3'		5'-TCC 3'	5'-AgT 3'	
			614		255		
			5'-Tgg 3'		5'-TCT 3'		
					299		
					5'-TCg 3'		
					616		
					5'-CgC 3'		
Well No.	25	26	27	28	29	30	31

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

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

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

101.421-06 – including *Taq* polymerase, IFU-01
101.421-06u – without *Taq* polymerase, IFU-02

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Lot-specific Information

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CELL LINE VALIDATION SHEET																				
HLA-A*23 SSP subtyping kit ²																				
				Well																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Lot No.:	201211401	201189702	201189703	201433204	201433205	201189706	201433207	201433208	201189709	201211410	201433211	201433212	201189713	201211414	201211415	201189716
	IHWC cell line ¹	A*	A*																	
1	9001 SA	*24:02		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
2	9280 LK707	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373	*30:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM	*02:01	*26:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL	*26:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025 DEU	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*26:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3	*24:02		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
11	9051 PITOUT	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*24:02		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
16	9037 SWEIG007	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540	*03:01	*80:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*33:03	*74:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*02:17		+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	W
22	9056 KOSE	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*02:01	*34:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*32:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*33:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*02:05		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*24:10	*29:01	-	-	-	-	-	-	-	-	-	W	-	-	-	+	-	-	+
28	9320 BEL5GB	*02:01	*29:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*30:01	*68:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*30:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*32:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*02:06	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*02:17		+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	W
39	9315 CML	*01:01	*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*02:07	*30:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*02:07		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*02:06	*02:07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*66:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*23:01	*24:02	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
46	9013 SCHU	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*02:16	*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND	*02:01	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

101.421-06 – including *Taq* polymerase, IFU-01
101.421-06u – without *Taq* polymerase, IFU-02

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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 5, 9, 11, 13, 15, 19, 20 and 23 to 26, 29 to 31 were available. The specificities of the primers in primer solutions 3, 13, 15, 19, 20, 23 and 26 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 4, 5, 9, 24, 29 and 30 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 11, 25 and 31 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 3, 7, 8, 10, 13, 15, 17, 19, 22, 23 and 27 one or two 3'-primers were not possible to test, and in primer solutions 19, 20 and 23 one or three 5'-primers were not possible to test. Additional primers in primer solutions 7, 8, 10 and 16 were tested by separately adding one additional 5'-primer and/or one additional 3'-primer.

101.421-06 – including *Taq* polymerase, IFU-01
101.421-06u – without *Taq* polymerase, IFU-02

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Lot No.: **59V**

Lot-specific Information

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ADDRESSES:

Manufacturer:

Olerup SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.

Tel: +46-8-717 88 27

Fax: +46-8-717 88 18

E-mail: info-ssp@olerup.com

Web page: <http://www.olerup-ssp.com>

Distributed by:

Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.

Tel: +43-1-710 15 00

Fax: +43-1-710 15 00 10

E-mail: support-at@olerup.com

Web page: <http://www.olerup.com>

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382

Tel: 1-877-OLERUP1

Fax: 610-344-7989

E-mail: info.us@olerup.com

Web page: <http://www.olerup.com>

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.