

Olerup SSP[®] HLA-A*34

Product number:	101.425-06u – without <i>Taq</i> polymerase
Lot number:	73M
Expiry date:	2014-April-01
Number of tests:	6
Number of wells per test:	8
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. 73M.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] HLA-A*34 LOT

The HLA-A*34 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP*[®] HLA-A*34 lot was made (Lot No. 23K).

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

Well	5'-primer	3'-primer	rationale
8	Added	Added	Added primer pair for the A*34:09 allele, exchanged positive control primer pair.

PRODUCT DESCRIPTION

HLA-A*34 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-A*34 group of alleles A*34:01 to A*34:09.

PLATE LAYOUT

Each test consists of 8 PCR reactions in an 8 well cut PCR plate.

1	2	3	4	5	6	7	8
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The 8 well cut PCR plate is marked with 'A34' in silver/gray ink.

Well No. 1 is marked with the Lot No. '73M'.

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 8 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

The interpretation of HLA-A*34 SSP subtypings will be influenced by three A*01, five A*02, five A*03, most A*11, four A*25, eleven A*26, three A*31, the A*32:15, most A*66 and the A*68:71 alleles when present on the other haplotype. In addition, the B*15:82 allele will be amplified by primer mix 5 and the C*07:81 allele will be amplified by primer mix 6.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*34 alleles, i.e. **A*34:01 to A*34:09 alleles**, recognized by the HLA Nomenclature Committee in July 2011¹ will give rise to unique amplification patterns by the primers in the HLA-A*34 subtyping kit.

The HLA-A*34 subtyping kit cannot distinguish the A*34:01:01-34:01:02 or the *34:02:01-34:02:02 alleles.

¹HLA-A alleles listed on the IMGT/HLA web page 2011-July-14, release 3.5.0, www.ebi.ac.uk/imgt/hla.

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Lot-specific information

www.olerup-ssp.com

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 11 alleles generate 9 amplification patterns that can be combined in 45 homozygous and heterozygous combinations. 23 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products were not considered in these calculations.

+++--+--+ *34:05, *34:08 = *34:05, *34:09
+++-----+ *34:01:01, *34:08 = *34:01:01, *34:09
++----+-- *34:01:01, *34:05 = *34:05, *34:05
+-+---++ *34:06, *34:08 = *34:06, *34:09
+-+---+- *34:02:01, *34:06 = *34:03, *34:07 = *34:06, *34:07
+-+---+ *34:03, *34:08 = *34:03, *34:09
+-+---+ *34:04, *34:08 = *34:04, *34:09
+-+---+ *34:02:01, *34:04 = *34:04, *34:04
+-+---+ *34:02:01, *34:08 = *34:02:01, *34:09 = *34:08, *34:08 =
*34:08, *34:09
+-+---+- *34:03, *34:06 = *34:06, *34:06

*34:01:01 = *34:01:01-34:01:02

*34:02:01 = *34:02:01-34:02:02

SPECIFICITY TABLE

HLA-A*34 SSP subtyping

Specificities and sizes of the PCR products of the 8 primer mixes used for HLA-A*34 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*34 alleles	Other amplified HLA Class I alleles ³
1⁴	100 bp	800 bp	*34:01:01-34:06, 34:08	*01:13, 01:17, 03:63, 03:88, 11:01:01-11:11, 11:13-11:16, 11:20-11:27, 11:29-11:39, 11:41-11:95, 11:97, 11:99N-11:105, 25:02, 26:13, 26:19, 26:33, 66:01, 66:04, 66:06-66:11, 66:13-66:14
2⁴	110 bp	1070 bp	*34:01:01-34:01:02, 34:05	*26:48, 26:69
3	195 bp	1070 bp	*34:02:01-34:02:02, 34:04, 34:07-34:09	
4^{4,5}	135 bp	800 bp	*34:03, 34:06	*03:01:19, 25:09, 26:14, 26:18, 26:28, 31:03-31:04
5	200 bp	800 bp	*34:04	*31:01:07, B*15:82
6⁶	155 bp	1070 bp	*34:05	*02:91, 02:322, 03:94, C*07:81
7^{4,7}	140 bp, 215 bp	1070 bp	*34:06-34:07	*11:96, 26:18, 31:03-31:04
8⁸	200 bp, 360 bp	800 bp	*34:08-34:09	*01:51, 02:55, 02:135, 02:309, 03:01:19, 03:24, 25:03, 25:13, 26:20, 26:30, 26:65, 31:04, 32:15, 66:02-66:03, 66:12, 66:16, 68:71

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*34 SSP typings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective lengths of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

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.

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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 two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band. In addition, wells number 4, 5 and 8 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

In the presence of a specific amplification the intensity of the control band often decreases.

³Due to the sharing of sequence motifs between HLA-A alleles non-HLA-A*34 alleles will be amplified by primer mixes 1, 2 and 4 to 8. In addition, the B*15:82 allele will be amplified by primer mix 5 and the C*07:81 allele will be amplified by primer mix 6.

⁴Specific PCR fragments shorter than 150 base pairs have a lower intensity than longer PCR bands.

⁵Primer mix 4 may give a lower yield of specific PCR product than the other A*34 primer mixes.

⁶Primer mix 6 may give rise to nonspecific amplifications.

⁷Primer mix 7: Specific PCR fragment of 140 bp in the A*34:06 and the A*26:18 and 31:03-31:04 alleles. Specific PCR fragment of 215 bp in the A*34:07 and the A*11:96 alleles.

⁸Primer mix 8: Specific PCR fragment of 200 bp in the A*34:08 and the A*01:51, 02:55, 03:24, 25:03, 26:20, 32:15 and 68:71 alleles. Specific PCR fragment of 360 bp in the A*34:09 and the A*02:135, 02:309, 03:01:19, 25:13, 26:30, 26:65, 31:04, 66:02-66:03, 66:12 and 66:16 alleles.

INTERPRETATION TABLE								
HLA-A*34 SSP subtyping								
Amplification patterns of the HLA-A*34:01 to 34:09 alleles								
	Well ⁴							
	1	2	3	4	5	6	7	8
Length of spec.	100	110	195	135	200	155	140	200
PCR product							215	360
Length of int.	800	1070	1070	800	800	1070	1070	800
pos. control ¹								
5'-primer(s) ²	282	270	363	423	78	445	103	102
	5'-CAg	3'5'-AAA	3'5'-ATA	3'5'-gCT	3'5'-TCC	3'5'-TCT	3'5'-CCT	3'5'-ACA
							423	341
							5'-gCT	3'5'-ggC
3'-primer(s) ³	341	341	517	517	238	559	277	259
	5'-CgT	3'5'-CgT	3'5'-CgT	3'5'-CgC	3'5'-CCT	3'5'-CgT	3'5'-ggA	3'5'-gTT
							524	418
							5'-CAC	3'5'-gTC
Well No.	1	2	3	4	5	6	7	8
HLA-A allele								
*34:01:01-34:01:02	1	2						
*34:02:01-34:02:02	1		3					
*34:03	1			4				
*34:04	1		3		5			
*34:05	1	2				6		
*34:06	1			4			7	
*34:07			3				7	
*34:08	1		3					8
*34:09			3					8
*01:13, 01:17, 03:63, 03:88, 11:01:01-11:11, 11:13-11:16, 11:20-11:27, 11:29-11:39, 11:41- 11:95, 11:97, 11:99N-11:105, 25:02, 26:13, 26:19, 26:33, 66:01, 66:04, 66:06-66:11, 66:13-66:14	1							
Well No.	1	2	3	4	5	6	7	8

Lot No.: **73M**

Lot-specific information

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Length of spec.	100	110	195	135	200	155	140	200
PCR product							215	360
Well No.	1	2	3	4	5	6	7	8
*01:51, 02:55, 02:135, 02:309, 03:24, 25:03, 25:13, 26:20, 26:30, 26:65, 32:15, 66:02- 66:03, 66:12, 66:16, 68:71								8
*02:91, 02:322, 03:94, C*07:81						6		
*03:01:19				4				8
*11:96							7	
*25:09, 26:14, 26:28				4				
*26:18, 31:03				4			7	
*26:48, 26:69		2						
*31:01:07, B*15:82					5			
*31:04				4			7	8
HLA-A allele								
Well No.	1	2	3	4	5	6	7	8

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band. In addition, wells number 4, 5 and 8 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

²The nucleotide position, in the 2nd or 3rd exons, 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, in the 2nd or 3rd exons, 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.

⁴Primer mix 7: Specific PCR fragment of 140 bp in the A*34:06 and the A*26:18 and 31:03-31:04 alleles. Specific PCR fragment of 215 bp in the A*34:07 and the A*11:96 alleles.

Primer mix 8: Specific PCR fragment of 200 bp in the A*34:08 and the A*01:51, 02:55, 03:24, 25:03, 26:20, 32:15 and 68:71 alleles. Specific PCR fragment of 360 bp in the A*34:09 and the A*02:135, 02:309, 03:01:19, 25:13, 26:30, 26:65, 31:04, 66:02-66:03, 66:12 and 66:16 alleles.

CELL LINE VALIDATION SHEET											
HLA-A*34 SSP subtyping kit											
				Well							
				1	2	3	4	5	6	7	8
				201073901	201073902	201073903	201073904	201073905	201073906	201073907	201191008
	IHWC cell line	A*	A*	Lot No.:							
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		-	-	-	-	-	-	-	-
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	-	-	-	-	-	-	-	-
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		-	-	-	-	-	-	-	-
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	+	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-A*34 SSP

Product number: 101.425-06u – without *Taq* polymerase
Lot number: 73M
Expiry date: 2014-April-01
Number of tests: 6
Number of wells per test: 8

Well specifications:

Well No.	Production No.
1	2010-739-01
2	2010-739-02
3	2010-739-03
4	2010-739-04
5	2010-739-05
6	2010-739-06
7	2010-739-07
8	2011-910-08

The specificity of each primer solution of the kit has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 4, 5 and 7 were available. The specificities of the primers in primer solutions 4 and 7 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 5 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solution 7 one of the 3'-primers was not possible to test. One additional 5'-primer and one additional 3'-primer in primer mix 8 were tested by separately adding one additional 3'-primer or 5'-primer.

Results: No false positive or false negative amplifications were obtained.

Date of approval: 2011-October-28

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP™ HLA-A*34
Product number: 101.425-06u
Lot number: 73M

Intended use: HLA-A*34 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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SE-112 51 Stockholm, Sweden
Phone: +46-8-717 88 27
Fax: +46-8-717 88 18

We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2008 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, conformity assessed using Annex IV, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at *Olerup* SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom. (Notified Body number: 0088.)

Stockholm, Sweden
2011-October-28

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

Lot No.: **73M**

Lot-specific information

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