

Lot No.: **28F**

Lot-specific information

***Olerup SSP*[®] HLA-A*66**

Product number: 101.427-06u – without *Taq* polymerase
Lot number: 28F
Expiry date: 2010-October-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. 28F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] HLA-A*66 LOT

The HLA-A*66 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP*[®] HLA-A*66 lot was made (Lot No. Y03).

The primers of the well detailed below has been exchanged, added or modified compared to the previous kit.

Well	5'-primer	3'-primer	rationale
4	Modified	-	Primer modified for the A*3402 allele.
5	-	Added	Primer added for the A*6607 allele.
6	Modified	-	Increased yield of specific PCR product
7	-	Modified	Increased specificity of specific primer pair.
8	Added	Added	Primer pair added for the A*6607 allele.

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PRODUCT DESCRIPTION

HLA-A*66 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-A*6601 to A*6607 alleles..

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 'A66' in silver/gray ink.

Well No. 1 is marked with the Lot No. '28F'.

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*66 SSP subtypings will be influenced by the A*01 alleles, several A*02 alleles, the A*11 alleles, two A*24 alleles, the A*25 alleles, the A*26 alleles, two A*31 allele, the A*3313, the A*34 alleles, the A*36, the A*4301 allele, most A*68 alleles, the A*6901 and the A*7410 allele when present on the other haplotype.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*66 alleles, i.e. **A*6601 to A*6607 alleles**, recognized by the HLA Nomenclature Committee in July 2008¹ will give rise to unique amplification patterns by the primers in the HLA-A*66 subtyping kit.

¹HLA-A alleles listed on the IMGT/HLA web page 2008-July-11, release 2.22.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 7 HLA-A*66 alleles can be combined in 28 homozygous and heterozygous combinations. Seventeen of these genotypes do not give rise to unique amplification patterns.

+++++--+ 6602,6607 = 6606,6607
+++++--- 6602,6604 = 6604,6606
++++-+-- 6601,6603 = 6603,6606
++++-+-- 6601,6602 = 6601,6606 = 6602,6606 = 6606,6606
+++-+--+ 6601,6607 = 6604,6605 = 6604,6607 = 6605,6607 =
6607,6607
+++-+--- 6601,6604 = 6604,6604

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SPECIFICITY TABLE

HLA-A*66 SSP subtyping

Specificities and sizes of the PCR products of the 8 primer mixes used for HLA-A*66 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*66 alleles	Other amplified HLA-A alleles ³
1⁵	175 bp	800 bp	6601, 6604-6607	9235, 250101-2505, 260101-2603, 2605-2608, 2610-2628, 2630-2633, 2635-2637, 4301
2⁴	100 bp	1070 bp	6601, 6604, 6606, 6607	0113, 0117, 110101-1111, 1113-1116, 1120-1127, 1129-1136, 2502, 2613, 2619, 2633, 340101 ^{weakly} , 340102-3404, 3405 ^{weakly} , 3406, 3408
3	425 bp	1070 bp	6601, 6602, 6604, 6606, 6607	0234-023503, 0256, 0262, 0278, 9203, 2419, 340101-3408, 680101-680202, 6806-6814, 6816-6819, 6821-6830, 6832-6843, 6901
4	165 bp	1070 bp	6602, 6603, 6606	3103, 3104, 3402-3404, 3406-3408
5^{4,6}	70, 100 bp	800 bp	6604, 6607	0255, 2603, 2606, 2621, 2630, 6805, 6815, 6820
6⁴	80 bp	800 bp	6601, 6604-6607	0113, 0128, 110101-1111, 1113-1116, 1119-1127, 1129-1136, 2419, 2444, 2603, 2606, 2621, 3103, 340101-3408
7	560 bp	1070 bp	6603	0216, 9231
8⁴	95 bp	1070 bp	6605, 6607	01010101-0104N, 0106, 0108-0112, 0114-0116N, 0118N-0130, 1117, 1119, 2444, 250101-250102, 2503-2506, 260101-2612, 2614-2618, 2620-2629, 2631, 2632, 2634-2637, 3103, 3313, 3601-3604, 4301, 7410, 8001

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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*66 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 length 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.

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 order to help in the correct orientation of the HLA-A*66 subtyping.

In addition, wells number 5 and 6 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*66 alleles will be amplified by primer mixes 1 to 8.

⁴Specific PCR fragments shorter than 125 base pairs have a lower intensity than longer PCR bands.

⁵Primer mix 1 may give rise to a PCR fragment approx. 500 bp in size. This band should be disregarded in the interpretation of HLA-A*66 subtypings.

⁶Primer mix 5: Specific PCR fragment of 70 bp in the A*0255, 2603, 2606, 2621, 2630, 6805, 6815 and 6820 alleles. Specific PCR fragment of 100 bp in the A*6604 and A*6607 alleles.

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INTERPRETATION TABLE								
HLA-A*66 SSP subtyping								
Amplification patterns of the HLA-A*6601 to 6607 alleles								
	Well⁴							
	1	2	3	4	5	6	7	8
Length of spec.	175	100	425	165	70	80	560	95
PCR product(s)					100			
Length of int.	800	1070	1070	1070	800	800	1070	1070
pos. control¹								
5'-primer(s)²	418	282	28	423	261	302	282	282
	5'-AgA ^{3'}	5'-CAg ^{3'}	5'-TCg ^{3'}	5'-gCT ^{3'}	5'-AAC ^{3'}	5'-ggA ^{3'}	5'-CAC ^{3'}	5'-CAC ^{3'}
	423				517			517
	5'-gCT ^{3'}				5'-AgA ^{3'}			5'-AgA ^{3'}
3'-primer(s)³	559	341	282	559	292	341	559	341
	5'-CCg ^{3'}	5'-CgT ^{3'}	5'-gAC ^{3'}	5'-CTC ^{3'}	5'-gTg ^{3'}	5'-CgT ^{3'}	5'-CTC ^{3'}	5'-CgT ^{3'}
					566			566
					5'-CCg ^{3'}			5'-CCg ^{3'}
					583			
					5'-gTg ^{3'}			
Well No.	1	2	3	4	5	6	7	8
HLA-A allele								
*6601	1	2	3			6		
*6602			3	4				
*6603				4			7	
*6604	1	2	3		5	6		
*6605	1					6		8
*6606	1	2	3	4		6		
*6607	1	2	3		5	6		8
*01010101-0104N, 0106, 0108-0112, 0114-0116N, 0118N-0127N, 0129, 0130, 1117, 2506, 2604, 2609, 2629, 2634, 3313, 3601-3604, 7410, 8001								8
*0113, 110101-1111, 1113-1116, 1120-1127, 1129-1136		2				6		
*0117		2						
*0128, 1119, 2444						6		8
*0216, 9231							7	
*0234-023503, 0256, 0262, 0278, 9203, 680101-680202, 6806-6814, 6816-6819, 6821-6830, 6832-6843, 6901			3					
Well No.	1	2	3	4	5	6	7	8

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Length of spec.	175	100	425	165	70	80	560	95
PCR product(s)					100			
Well No.	1	2	3	4	5	6	7	8
*0255, 6805, 6815, 6820					5			
*9235	1							
*2419	3				6			
*250101-250102, 2503-2505, 260101-2602, 2605, 260701- 2608, 2610-2612, 2614-2618, 2620, 2622-2628, 2631, 2632, 2635-2637, 4301	1							8
*2502, 2613, 2619, 2633	1	2						
*2603, 2606, 2621	1				5	6		8
*2630	1				5			
*3103				4		6		8
*3104				4				
*340101, 3405		w	3			6		
*340102	2		3		6			
*3402-3404, 3406, 3408		2	3	4		6		
*3407	3			4	6			
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 order to help in the correct orientation of the HLA-A*66 subtyping. .

In addition, wells number 5 and 6 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 1st, 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 5: Specific PCR fragment of 70 bp in the A*0255, 2603, 2606, 2621, 2630, 6805, 6815 and 6820 alleles. Specific PCR fragment of 100 bp in the A*6604 and A*6607 alleles.

'w', might be weakly amplified.

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CELL LINE VALIDATION SHEET												
HLA-A*66 SSP subtyping kit												
					Well							
					1	2	3	4	5	6	7	8
				Lot No.:	200853101	200853102	200853103	200853104	200853105	200853106	200853107	200853108
	IHWC cell line	A*	A*									
1	9001 SA	*2402			-	-	-	-	-	-	-	-
2	9280 LK707	*0201			-	-	-	-	-	-	-	-
3	9011 E4181324	*0101			-	-	-	-	-	-	-	+
4	9275 GU373	*3001			-	-	-	-	-	-	-	-
5	9009 KAS011	*0101			-	-	-	-	-	-	-	+
6	9353 SM	*0201	*2603		+	-	-	-	+	+	-	+
7	9020 QBL	*2601			+	-	-	-	-	-	-	+
8	9007 DEM	*0201			-	-	-	-	-	-	-	-
9	9026 YAR	*2601			+	-	-	-	-	-	-	+
10	9107 LKT3	*2402			-	-	-	-	-	-	-	-
11	9051 PITOUT	*2902			-	-	-	-	-	-	-	-
12	9052 DBB	*0201			-	-	-	-	-	-	-	-
13	9067 BTB	*0201			-	-	-	-	-	-	-	-
14	9071 OLGA	*3101			-	-	-	-	-	-	-	-
15	9075 DKB	*2402			-	-	-	-	-	-	-	-
16	9037 SWEIG007	*2902			-	-	-	-	-	-	-	-
17	9008 WILJON	*2501			+	-	-	-	-	-	-	+
18	9257 32367	*3303	*7401		-	-	-	-	-	-	-	-
19	9038 BM16	*0201			-	-	-	-	-	-	-	-
20	9059 SLE005	*0201			-	-	-	-	-	-	-	-
21	9064 AMALA	*0217			-	-	-	-	-	-	-	-
22	9056 KOSE	*0201			-	-	-	-	-	-	-	-
23	9124 IHL	*0201	*3401		-	+	+	-	-	+	-	-
24	9035 JBUSH	*3201			-	-	-	-	-	-	-	-
25	9049 IBW9	*3301			-	-	-	-	-	-	-	-
26	9285 WT49	*0205			-	-	-	-	-	-	-	-
27	9191 CH1007	*2410	*2901		-	-	-	-	-	-	-	-
28	9320 BEL5GB	*0201	*2902		-	-	-	-	-	-	-	-
29	9050 MOU	*2902			-	-	-	-	-	-	-	-
30	9021 RSH	*3001	*6802		-	-	+	-	-	-	-	-
31	9019 DUCAF	*3002			-	-	-	-	-	-	-	-
32	9297 HAG	*0201			-	-	-	-	-	-	-	-
33	9098 MT14B	*3101			-	-	-	-	-	-	-	-
34	9104 DHIF	*3101			-	-	-	-	-	-	-	-
35	9302 SSTO	*3201			-	-	-	-	-	-	-	-
36	9024 KT17	*0206	*1101		-	+	-	-	-	+	-	-
37	9065 HHKB	*0301			-	-	-	-	-	-	-	-
38	9099 LZL	*0217			-	-	-	-	-	-	-	-
39	9315 CML	*0101	*0301		-	-	-	-	-	-	-	+
40	9134 WHONP199	*0207	*3001		-	-	-	-	-	-	-	-
41	9055 H0301	*0301			-	-	-	-	-	-	-	-
42	9066 TAB089	*0207			-	-	-	-	-	-	-	-
43	9076 T7526	*0207			-	-	-	-	-	-	-	-
44	9057 TEM	*6601			+	+	+	-	-	+	-	-
45	9239 SHJO	*2301	*2402		-	-	-	-	-	-	-	-
46	9013 SCHU	*0301			-	-	-	-	-	-	-	-
47	9045 TUBO	*0216	*0301		-	-	-	-	-	-	+	-
48	9303 TER-ND	*0201	*1101		-	+	-	-	-	+	-	-

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CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-A*66 SSP

Product number: 101.427-06u – without *Taq* polymerase
Lot number: 28F
Expiry date: 2010-October-01
Number of tests: 6
Number of wells per test: 8

Well specifications:

Well No.	Production No.
1	2008-531-01
2	2008-531-02
3	2008-531-03
4	2008-531-04
5	2008-551-05
6	2008-531-06
7	2008-531-07
8	2008-531-08

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

The specificities of additional primers in primer solution 5 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. One of the 3'-primers in primer mixes 5 and 8 were not possible to test.

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

Date of approval: 2009-May-25

Approved by:

Quality Control, Supervisor

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Declaration of Conformity

Product name: *Olerup* SSP® HLA-A*66
Product number: 101.427-06u
Lot number: 28F

Intended use: HLA-A*66 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Hasselstigen 1
SE-133 33 Saltsjöbaden, 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 9001:2000 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, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

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

Saltsjöbaden, Sweden
2009-May-25

Olle Olerup
Managing Director

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