

Lot No.: **65E**

Lot-specific information

***Olerup* SSP[®] HLA-A*74**

Product number:	101.433-06u – without <i>Taq</i> polymerase
Lot number:	65E
Expiry date:	2010-May-01
Number of tests:	6
Number of wells per test:	13
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. 65E.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP[®] HLA-A*74 Lot

The HLA-A*74 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup* SSP[®] HLA-A*74 lot was made (Lot No. X45).

The HLA-A*74 primer set is unchanged compared to the previous lot.

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PRODUCT DESCRIPTION

HLA-A*74 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*7401 to A*7412N alleles.

PLATE LAYOUT

Each test consists of 13 PCR reactions in a 16 well cut PCR plate. Wells 14 to 16 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	empty	empty	empty

The 16 well cut PCR plate is marked with 'A*74'.

Well No. 1 is marked with the Lot No. '65E'.

The PCR plates are covered with a PCR-compatible foil.

Please note: When removing each 16 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

The interpretation of HLA-A*74 SSP subtypings will be influenced by two A*02, two A*03, A*260702, the A*29, the A*32, two A*33 and the A*6830 alleles when present on the other haplotype.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*74 alleles, i.e. **A*7401 to A*7412N alleles**, recognized by the HLA Nomenclature Committee in April 2008¹ will give rise to unique amplification patterns by the primers in the HLA-A*74 subtyping kit.

¹HLA-A alleles listed on the IMGT/HLA web page 2008-April-08, release 2.21.0, www.ebi.ac.uk/imgt/hla.

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RESOLUTION IN HOMO- AND HETEROZYGOTES

The 12 HLA-A*74 alleles can be combined in 78 homozygous and heterozygous combinations. Eighteen of these genotypes do not give rise to unique amplification patterns.

++-+-----	-----	7401, 7404 = 7404, 7404
++-+-----	-----	7401, 7405 = 7405, 7405
++-+-----	-----	7401, 7406 = 7406, 7406
++-----+	-----	7401, 7408 = 7408, 7408
++-----+	+-----	7401, 7403 = 7403, 7403
++-----+	-+---	7401, 7409 = 7409, 7409
++-----+	---+-	7401, 7411 = 7411, 7411
++-----+	-----+	7401, 7412N = 7412N, 7412N
+--+-----	-----	7402, 7407 = 7407, 7407

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SPECIFICITY TABLE

HLA-A*74 SSP subtyping

Specificities and sizes of the PCR products of the 13 primer mixes used for HLA-A*74 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*74 alleles	Other amplified HLA-A alleles ³
1	160 bp	800 bp	7401-7409, 7411, 7412N	
2	285 bp	1070 bp	7401, 7403, 7404-7406 [?] , 7408-7412N [?]	
3	285 bp	1070 bp	7402, 7404-7406 [?] , 7407, 7408-7412N [?]	29010101-2903, 2904-2910 [?] , 2911, 2912-2917 [?] , 320101-3202, 3203 [?] , 3205, 3206-3216 [?]
4⁴	135 bp	1070 bp	7404	020109, 023503, 030103 ^{weakly} , 0323, 260702, 3308, 3309, 6830
5	230 bp	800 bp	7405	
6	155 bp	800 bp	7406	
7	150 bp	800 bp	7407	
8⁴	115 bp	1070 bp	7408	
9	170 bp	800 bp	7403	
10	225 bp	1070 bp	7409	
11	200 bp	800 bp	7410	
12⁴	130 bp	1070 bp	7411	
13	215 bp	1070 bp	7412N	

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*74 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.

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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*74 subtyping.

In addition, wells number 5, 6, 7, 9 and 11 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 a few non-HLA-A*74 alleles will be amplified by primer mixes 3 and 4.

⁴Short specific PCR fragments are less intense and not as sharp as longer specific bands.

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

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INTERPRETATION TABLE								
HLA-A*74 SSP subtyping								
Amplification patterns of the A*7401 to 7412N alleles								
	Well							
	1	2	3	4	5	6	7	8
Length of spec.	160	285	285	135	230	155	150	115
PCR product								
Length of int.	800	1070	1070	1070	800	800	800	1070
pos. control¹								
5'-primer²	180	67	67	144	98	180	180	448
	5'-TTT ^{3'}	5'-CCA ^{3'}	5'-CCT ^{3'}	5'-gCC ^{3'}	5'-CTT ^{3'}	5'-TTT ^{3'}	5'-TTT ^{3'}	5'-CCT ^{3'}
3'-primer(s)³	299	180	180	240	289	292	290	523
	5'-CCA ^{3'}	5'-TCA ^{3'}	5'-TCA ^{3'}	5'-ggA ^{3'}	5'-AgC ^{3'}	5'-gTg ^{3'}	5'-CAA ^{3'}	5'-ACT ^{3'}
	299							
	5'-CCA ^{3'}							
Well No.	1	2	3	4	5	6	7	8
HLA-A allele								
*7401	1	2						
*7402	1		3					
*7403	1	2						
*7404	1	?	?	4				
*7405	1	?	?		5			
*7406	1	?	?			6		
*7407	1		3				7	
*7408	1	?	?					8
*7409	1	?	?					
*7410		?	?					
*7411	1	?	?					
*7412N	1	?	?					
*020109, 023503, 0323, 260702, 3308, 3309, 6830				4				
*030103				W				
*29010101-2903, 2911, 320101-3202, 3205			3					
*2904-2910, 2912-2917, 3203, 3206-3216			?					
HLA-A allele								
Well No.	1	2	3	4	5	6	7	8

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INTERPRETATION TABLE					
HLA-A*74 SSP subtyping					
Amplification patterns of the A*74 alleles					
Well					
9	10	11	12	13	
170	225	200	130	215	Length of spec.
					PCR product
800	1070	800	1070	1070	Length of int.
					pos. control¹
180	113	180	211	364	5'-primer²
5'-TTT ^{3'}	5'-CCC ^{3'}	5'-TTT ^{3'}	5'-AGT ^{3'}	5'-TgA ^{3'}	
308	299	341	299	538	3'-primer(s)³
5'-TCg ^{3'}	5'-CCA ^{3'}	5'-CgT ^{3'}	5'-CCA ^{3'}	5'-CAA ^{3'}	
9	10	11	12	13	Well No.
					HLA-A allele
					*7401
					*7402
9					*7403
					*7404
					*7405
					*7406
					*7407
					*7408
	10				*7409
		11			*7410
			12		*7411
				13	*7412N
					*020109, 023503, 0323, 260702, 3308, 3309, 6830
					*030103
					*29010101-2903, 2911, 320101-3202, 3205
					*2904-2910, 2912-2917, 3203, 3206-3216
					HLA-A allele
9	10	11	12	13	Well No.

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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*74 subtyping. .

In addition, wells number 5, 6, 7, 9 and 11 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.

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

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CELL LINE VALIDATION SHEET																		
HLA-A*74 SSP subtyping kit																		
					Well													
					1	2	3	4	5	6	7	8	9	10	11	12	13	
				Lot No.:	200413401	200315901	200413403	200315904	200315905	200315906	200315907	200413408	200315909	200413410	200413411	200730912	200618213	
	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*74 SSP

Product number: 101.433-06u – without *Taq* polymerase
Lot number: 65E
Expiry date: 2010-May-01
Number of tests: 6
Number of wells per test: 13

Well specifications:

Well No.	Production No.	Well No.	Production No.
1	2004-134-01	9	2003-159-09
2	2003-159-02	10	2004-134-10
3	2004-134-03	11	2004-134-11
4	2003-159-04	12	2007-309-12
5	2003-159-05	13	2006-182-13
6	2003-159-06		
7	2003-159-07		
8	2004-134-08		

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 to 13 were available. The specificities of the primers in primer solutions 4, 6, 7 and 11 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 5, 8, 10 and 12 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 10 and 13 it was only possible to test the 3'-primer, the 5'-primer was not possible to test.

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

Date of approval: 2009-May-27

Approved by:

Quality Control, Supervisor

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Declaration of Conformity

Product name: Olerup SSP® HLA-A*74
Product number: 101.433-06u
Lot number: 65E

Intended use: HLA-A*74 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-27

Olle Olerup
Managing Director

Lot No.: **65E**

Lot-specific information

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