

Olerup SSP® HLA-A*01

Product number:	101.411-24/06– including <i>Taq</i> pol.
Lot number:	94E
Expiry date:	2010-August-01
Number of tests:	24 test – Product No. 101.411-24 6 tests – Product No. 101.411-06
Number of wells per test:	24
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. 94E.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP® HLA-A*01 Lot

The HLA-A*01 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP®* HLA-A*01 lot was made (**Lot No. X98**).

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	Primer added for the A*01010102N allele.
10	Added	Added	Primer pair added for the A*0129 allele.
11	Added	Added	Primer pair added for the A*0128 allele.
19	Added	Added	Primer pair added for the A*0130 allele.
23	Added	Added	Primer pair added for the A*0130 allele.

Changes in revision R01 compared to R00:

1. Nucleotide position for downstream primer in primer mix 20 corrected to position 545 in the Interpretation Table.

PRODUCT DESCRIPTION

HLA-A*01 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*0101 to A*0130 alleles.

PLATE LAYOUT

Each 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	24

The 24 well cut PCR plate is marked with 'HLA-A*01'.

Well No. 1 is marked with '94E'.

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

The interpretation of HLA-A*01 SSP subtypings will be influenced by four A*02 alleles, the A*03 alleles, the A*11 alleles, one A*23 alleles, four A*24 alleles, the A*2632 allele, most A*30 alleles, two A*31 alleles, one A*32 allele, one A*33 allele, five A*34 alleles, the A*36 alleles, the A*7410 allele and the A*8001 allele when present on the other haplotype.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*01 allele¹, i.e. **A*0101 to A*0130**, recognized by the HLA Nomenclature Committee in July 2008¹ will give rise to unique amplification patterns by the primers in the HLA-A*01 subtyping kit.

The HLA-A*01 subtyping kit cannot distinguish the A*01010101, A*010102 to A*010105 alleles.

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 29 HLA-A*01 alleles generate 30 amplification patterns that can be combined in 465 homozygous and heterozygous combinations. 210 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products generated by primer mixes 8, 10, 11, 16, 20 and 23 were considered in these calculations.

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Lot-specific Information

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+++-----+	-----	-+---+---	0107,0120 = 0120,0123
+++-----+	-----	-+-----	0102,0107 = 0102,0123
+++-----	+-----	-+-----	0102,0108 = 0102,0127N
+++-----	--+-----	-+---+---	0111N,0120 = 0120,0128
+++-----	--+-----	-+-----	0102,0111N = 0102,0128
+++-----+	-----	-+-----	0103,0107 = 0103,0123
+++-----	--+-----	-+-----	0103,0111N = 0103,0128
+++-----+	-----	-+-----	0104N,0107 = 0104N,0123
+++-----	+-----	-+-----	0104N,0108 = 0104N,0127N
+++-----	--+-----	-+-----	0104N,0111N = 0104N,0128
+++-----	-----	-+-----	0101,0104N = 0104N,0104N
+++-----+	-----	-+-----	0107,0109 = 0109,0123
+++-----+	+-----	-+-----	0108,0109 = 0109,0127N
+++-----	--+-----	-+-----	0109,0111N = 0109,0128
+++-----	-----	-+-----	0101,0109 = 0109,0109
+++-----+	-----	-+-----	0106,0107 = 0106,0123
+++-----	+-----	-+-----	0106,0108 = 0106,0127N
+++-----	--+-----	-+-----	0106,0111N = 0106,0128
+++-----	-----	-+-----	0101,0106 = 0106,0106
+++-----+	+-----+	-+-----	01010102N,0108 = 01010102N,0127N
+++-----+	+-----	-+-----	0107,0108 = 0107,0127N = 0108,0123 = 0123,0127N
+++-----+	-+-----	-+---+---	0107,0110 = 0110,0123
+++-----+	-+-----	-+-----	0107,0129 = 0123,0129
+++-----+	--+-----+	-+-----	01010102N,0111N = 01010102N,0128
+++-----+	--+-----	-+-----	0107,0111N = 0111N,0123 = 0123,0128
+++-----+	-----+--	-+---+---	0107,0119 = 0119,0123
+++-----+	-----+--	-+---+---	0107,0112 = 0112,0123
+++-----+	-----+--	-+---+---	0107,0121 = 0121,0123
+++-----+	-----+--	-+-----	0107,0117 = 0117,0123
+++-----+	-----+--	-+-----	0107,0114 = 0114,0123
+++-----+	-----+--	-+-----	0101,01010102N = 01010102N,01010102N = 01010102N,0107 = 01010102N,0115N = 01010102N,0123 = 0107,0115N = 0115N,0123
+++-----+	-----	+++-----	0107,0116N = 0116N,0123
+++-----+	-----	-+---+---	0107,0130 = 0123,0130
+++-----+	-----	-+---+---	0107,0118N = 0118N,0123
+++-----+	-----	-+---+---	0107,0126 = 0123,0126
+++-----+	-----	-+---+---	0107,0122N = 0122N,0123
+++-----+	-----	-+---+---	0107,0124 = 0123,0124
+++-----+	-----	-+---+---	0107,0125 = 0123,0125
+++-----+	-----	-+---+---	0101,0107 = 0101,0123 = 0107,0123 = 0123,0123
+++-----	+++-----	-+---+---	0108,0110 = 0110,0127N
+++-----	+++-----	-+-----	0108,0129 = 0127N,0129
+++-----	+++-----	-+-----	0108,0113 = 0113,0127N
+++-----	+++-----	-+-----	0108,0111N = 0108,0128 = 0111N,0127N = 0127N,0128
+++-----	+---+---	-+---+---	0108,0121 = 0121,0127N
+++-----	+---+---	-+-----	0108,0117 = 0117,0127N
+++-----	+-----+	-+-----	0108,0115N = 0115N,0127N
+++-----	+-----	+++-----	0108,0116N = 0116N,0127N

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++----- +----- -+-+---- 0108,0130 = 0127N,0130
++----- +----- -+-+---- 0108,0118N = 0118N,0127N
++----- +----- -+-+---- 0108,0126 = 0126,0127N
++----- +----- -+-+---- 0108,0122N = 0122N,0127N
++----- +----- -+-+---- 0108,0124 = 0124,0127N
++----- +----- -+-+---- 0108,0125 = 0125,0127N
++----- +----- -+-+---- 0101,0108 = 0101,0127N = 0108,0127N =
0127N,0127N
++----- -+-+---- -+-+---- 0110,0111N = 0110,0128
++----- -+-+---- -+-+---- 0111N,0129 = 0128,0129
++----- -+-+---- -+-+---- 0110,0112 = 0110,0119 = 0119,0129
++----- -+-+---- -+-+---- 0110,0121 = 0121,0129
++----- -+-+---- -+-+---- 0101,0110 = 0110,0110 = 0110,0126 =
0110,0129 = 0126,0129
++----- -+-+---- -+-+---- 0101,0129 = 0129,0129
++----- -+-+---- -+-+---- 0111N,0119 = 0119,0128
++----- -+-+---- -+-+---- 0111N,0112 = 0112,0128
++----- -+-+---- -+-+---- 0111N,0121 = 0121,0128
++----- -+-+---- -+-+---- 0101,0113 = 0111N,0113 = 0111N,0117 =
0113,0117 = 0117,0128
++----- -+-+---- -+-+---- 0113,0113 = 0113,0128
++----- -+-+---- -+-+---- 0111N,0114 = 0114,0128
++----- -+-+---- -+-+---- 0111N,0115N = 0115N,0128
++----- -+-+---- -+-+---- 0111N,0116N = 0116N,0128
++----- -+-+---- -+-+---- 0111N,0130 = 0128,0130
++----- -+-+---- -+-+---- 0111N,0118N = 0118N,0128
++----- -+-+---- -+-+---- 0111N,0126 = 0126,0128
++----- -+-+---- -+-+---- 0111N,0122N = 0122N,0128
++----- -+-+---- -+-+---- 0111N,0124 = 0124,0128
++----- -+-+---- -+-+---- 0111N,0125 = 0125,0128
++----- -+-+---- -+-+---- 0101,0111N = 0101,0128 = 0111N,0111N
= 0111N,0128
++----- -+-+---- -+-+---- 0101,0119 = 0112,0121 = 0112,0126 =
0119,0121 = 0119,0125 = 0119,0126
++----- -+-+---- -+-+---- 0101,0112 = 0112,0125
++----- -+-+---- -+-+---- 0101,0121 = 0121,0121 = 0121,0126
++----- -+-+---- -+-+---- 0101,0117 = 0117,0117
++----- -+-+---- -+-+---- 0101,0115N = 0115N,0115N
++----- -+-+---- -+-+---- 0101,0116N = 0116N,0116N
++----- -+-+---- -+-+---- 0101,0130 = 0118N,0124 = 0118N,0130 =
0124,0130 = 0130,0130
++----- -+-+---- -+-+---- 0101,0118N = 0118N,0118N
++----- -+-+---- -+-+---- 0101,0126 = 0126,0126
++----- -+-+---- -+-+---- 0101,0122N = 0122N,0122N
++----- -+-+---- -+-+---- 0101,0124 = 0124,0124
++----- -+-+---- -+-+---- 0101,0125 = 0125,0125
-+-+---- -+-+---- -+-+---- 0112,0119 = 0114,0119 = 0119,0119
-+-+---- -+-+---- -+-+---- 0112,0112 = 0112,0114

0101 = 01010101, 010102-010105

SPECIFICITY TABLE

HLA-A*01 SSP subtyping

Specificities and sizes of the PCR products of the 24 primer mixes used for HLA-A*01 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*01 alleles	Other amplified HLA-A alleles ³
1	235 bp	800 bp	01010101-0102, 0104N, 0106, 0107, 0109-0111N, 0113, 0115N-0118N, 0121-0130	0318, 110101-1103, 1105-1125, 1129-1134, 1136, 3604
2⁴	145 bp	1070 bp	01010101-010105, 0103, 0104N, 0106-0117, 0118 ^{weakly} , 0119, 0121-0123, 0124 ^{weakly} , 0125-0130	3601-3604
3⁴	120 bp	800 bp	0102, 0120	
4	305 bp	1070 bp	0103	1126, 2632, 3313, 3603, 7410
5	470 bp	1070 bp	0104N	0321N, 1121N, 2307N, 2411N
6	210 bp	1070 bp	0109	
7⁶	215 bp	800 bp	0106	0305, 112401-1125, 1131, 1135, 300101-3003, 3007-3016, 3018-3020, 3022-3025, 3103, 3104, 3402-3404, 3407, 3408, 8001
8^{4,7}	110, 180 bp	1070 bp	01010102N, 0107, 0123	
9	235 bp	1070 bp	0108, 0127N	
10⁸	155, 270 bp	800 bp	0110, 0129	1114 ^{weakly} , 8001 ^{weakly}
11^{4,9}	135, 275 bp	800 bp	0111N, 0113, 0128	
12^{4,10}	85 bp	800 bp		3601-3604
13	205 bp	1070 bp	0112, 0119, 0121	0302, 0307 ^{weakly}

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				0310, 0331, 0332, 1131 ^{weakly} , 1135 ^{weakly} , 3004 ^{weakly} , 3006 ^{weakly} , 3009 ^{weakly} , 3017 ^{weakly} , 3103 ^{weakly} , 3104 ^{weakly}
14⁴	120 bp	1070 bp	0113, 0117	
15⁵	235 bp	1070 bp	0112, 0114, 0119	03010101-0317, 0319-0339, 1104, 1127, 1135, 2451, 300101-3004, 3006-3013, 3015- 3020, 3022-3025, 3103, 3104, 3204, 3402-3404, 3407, 3408, 3601, 3602
16¹¹	180, 235 bp	1070 bp	01010102N, 0115N	
17	210 bp	1070 bp	0116N	
18⁴	135 bp	1070 bp	01010101-0104N, 0106, 0108-0112, 0114-0127N, 0129, 0130	3601-3604
19	170 bp	1070 bp	0118N, 0130	2422
20^{4,12}	65, 425 bp	800 bp	0110, 0119, 0121, 0126	
21	255 bp	1070 bp	0120	0219, 0236, 0237, 0254, 2414
22	600 bp	1070 bp	0122N	
23¹³	155, 175 bp	1070 bp	0124, 0130	2422
24⁵	215 bp	1070 bp	0112, 0119, 0125	0302, 0310, 0331, 0332, 110101- 1107, 1109-1122, 1127, 1129, 1130, 1132-1134, 1136

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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-A*01 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.

² The 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*01 subtyping.

In addition, wells number 3, 7, 10 to 12 and 20 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.

³ Due to the sharing of sequence motifs between HLA-A alleles a few non-HLA-A*01 alleles will be amplified by primer mixes 1, 2, 4, 5, 7, 10, 12, 13, 15, 18, 19, 21, 23 and 24.

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

⁵ Primer mixes 15 and 24 may give rise to non-specific amplifications.

⁶ Primer mix 7 may give rise to primer oligomer formation.

⁷ Primer mix 8: Specific PCR fragment of 110 bp in the A*0107 and A*0123 alleles. Specific PCR fragment of 180 bp in the A*01010102N allele.

⁸ Primer mix 10: Specific PCR fragment of 155 bp in the A*0110 allele. Specific PCR fragment of 270 bp in the A*0129 allele.

⁹ Primer mix 11: Specific PCR fragment of 135 bp in the A*0128 allele. Specific PCR fragment of 275 bp in the A*0111N and A*0113 alleles.

¹⁰ Primer mix 12 might faintly amplify the A*0227, A*110101-110202, 1104-1107, 1109-1113, 1115-1119 and 1121N-1132 alleles.

¹¹ Primer mix 16: Specific PCR fragment of 180 bp in the A*01010102N allele. Specific PCR fragment of 235 bp in the A*0115N allele.

¹² Primer mix 20: Specific PCR fragment of 65 bp in the A*0110, A*0121 and A*0126 alleles. Specific PCR fragment of 425 bp in the A*0119 allele.

¹³ Primer mix 23: Specific PCR fragment of 155 bp in the A*0124 alleles. Specific PCR fragment of 175 bp in the A*0130 and *2422 alleles.

INTERPRETATION TABLE												
HLA-A*01 SSP subtyping												
Amplification patterns of the A*0101 to 0130 alleles												
	Well ⁵											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	235	145	120	305	470	210	215	110	235	155	135	85
PCR product								180		270	275	
Length of int.	800	1070	800	1070	1070	1070	800	1070	1070	800	800	800
pos. control ¹												
5'-primer(s) ²	363	98	123	341	3rd I	171	363	203	363	113	203	527
	5'-ATA ^{3'}	5'-CTT ^{3'}	5'-AgT ^{3'}	5'-ggA ^{3'}	5'-ATA ^{3'}	5'-TTA ^{3'}	5'-ATA ^{3'}	5'-gAA ^{3'}	5'-ATA ^{3'}	5'-CCC ^{3'}	5'-gAA ^{3'}	5'-TgC ^{3'}
										413	363	
										5'-CCg ^{3'}	5'-ATA ^{3'}	
3'-primer(s) ³	559	203	203	362	621	341	539	270	553	341	299	570
	5'-CCg ^{3'}	5'-TCT ^{3'}	5'-TCT ^{3'}	5'-TCA ^{3'}	5'-CCC ^{3'}	5'-CgT ^{3'}	5'-TCA ^{3'}	5'-ACA ^{3'}	5'-CTA ^{3'}	5'-CgT ^{3'}	5'-CCA ^{3'}	5'-CAC ^{3'}
								341	559	527	597	
								5'-CgT ^{3'}	5'-CCA ^{3'}	5'-CCC ^{3'}	5'-TTA ^{3'}	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-A allele ⁴												
*01010101, 010102-010105	1	2										
*01010102N	1	2						8				
*0102	1		3									
*0103		2		4								
*0104N	1	2			5							
*0106	1	2					7					
*0107	1	2						8				
*0108		2							9			
*0109	1	2				6						
*0110	1	2								10		
*0111N	1	2									11	
*0112		2										
*0113	1	2									11	
*0114		2										
*0115N	1	2										
*0116N	1	2										
*0117	1	2										
*0118N	1	w										
*0119		2										
*0120			3									
*0121	1	2										
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE												
HLA-A*01 SSP subtyping												
Amplification patterns of the A*0101 to 0130 alleles												
Well ⁵												
13	14	15	16	17	18	19	20	21	22	23	24	
205	120	235	180	210	135	170	65	255	600	155	215	Length of spec.
			235				425			175		PCR product
1070	1070	1070	1070	1070	1070	1070	800	1070	1070	1070	1070	Length of int.
												pos. control ¹
363	203	363	203	363	203	215	203	355	3 rd I	89	363	5'-primer(s) ²
5'-ATA ^{3'}	5'-gAA ^{3'}	5'-ATA ^{3'}	5'-gAA ^{3'}	5'-ATA ^{3'}	5'-gAA ^{3'}	5'-gCC ^{3'}	5'-gAA ^{3'}	5'-CCg ^{3'}	5'-ATA ^{3'}	5'-gAA ^{3'}	5'-ATA ^{3'}	
			363			413	521			413		
			5'-ATA ^{3'}			5'-CCA ^{3'}	5'-ggC ^{3'}			5'-CCA ^{3'}		
527	282	559	341	531	299	341	346	570	750	203	538	3'-primer(s) ³
5'-CCA ^{3'}	5'-gAC ^{3'}	5'-CgT ^{3'}	5'-CgT ^{3'}	5'-TCC ^{3'}	5'-TCg ^{3'}	5'-CgT ^{3'}	5'-AgC ^{3'}	5'-CCg ^{3'}	5'-T.g ^{3'}	5'-TCt ^{3'}	5'-CTg ^{3'}	
			557			545	545			545		
			5'-gC ^{3'}			5'-AgA ^{3'}	5'-AgA ^{3'}			5'-AgA ^{3'}		
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												HLA-A allele ⁴
					18							*01010101, 010102-010105
			16		18							*01010102N
					18							*0102
					18							*0103
					18							*0104N
					18							*0106
					18							*0107
					18							*0108
					18							*0109
					18		20					*0110
					18							*0111N
13		15			18						24	*0112
	14											*0113
		15			18							*0114
			16		18							*0115N
				17	18							*0116N
	14				18							*0117
					18	19						*0118N
13		15			18		20				24	*0119
					18			21				*0120
13					18		20					*0121
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

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Length of spec.	235	145	120	305	470	210	215	110	235	155	135	85
PCR product								180		270	275	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*0122N	1	2										
*0123	1	2						8				
*0124	1	w										
*0125	1	2										
*0126	1	2										
*0127N	1	2							9			
*0128	1	2									11	
*0129	1	2								10		
*0130	1	2										
*0219, 0236, 0237, 0254, 2414												
*03010101-030106, 0303N, 0304, 0306, 0308, 0309, 0311N- 0317, 0319, 0320, 0322- 0330, 0333-0339, 2451, 3204												
*0302, 0310, 0331, 0332												
*0305, 300101-3003, 3007, 3008, 3010-3013, 3015, 3016, 3018-3020, 3022-3025, 3402-3404, 3407, 3408							7					
*0307, 3004, 3006, 3017												
*0318, 1108, 1123	1											
*0321N					5							
*110101-1103, 1105- 1107, 1109-1113, 111501-1120, 1122, 1129, 1130, 1132-1134, 1136	1											
*1104, 1127												
*1114	1									w		
*1121N	1				5							
*112401-1125	1						7					
*1126, 2632, 3313, 7410				4								
*1131	1						7					
*1135, 3009, 3103, 3104							7					
*2307N, 2411N					5							
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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205	120	235	180	210	135	170	65	255	600	155	215	Length of spec. PCR product
			235				425				175	
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
					18				22			*0122N
					18							*0123
					18					23		*0124
					18						24	*0125
					18		20					*0126
					18							*0127N
												*0128
					18							*0129
					18	19				23		*0130
								21				*0219, 0236, 0237, 0254, 2414
		15										*03010101-030106, 0303N, 0304, 0306, 0308, 0309, 0311N- 0317, 0319, 0320, 0322- 0330, 0333-0339, 2451, 3204
13		15									24	*0302, 0310, 0331, 0332
		15										*0305, 300101-3003, 3007, 3008, 3010-3013, 3015, 3016, 3018-3020, 3022-3025, 3402-3404, 3407, 3408
w		15										*0307, 3004, 3006, 3017
												*0318, 1108, 1123
		15										*0321N
											24	*110101-1103, 1105- 1107, 1109-1113, 111501-1120, 1122, 1129, 1130, 1132-1134, 1136
		15									24	*1104, 1127
											24	*1114
											24	*1121N
												*112401-1125
												*1126, 2632, 3313, 7410
w												*1131
w		15										*1135, 3009, 3103, 3104
												*2307N, 2411N
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

Length of spec.	235	145	120	305	470	210	215	110	235	155	135	85
PCR product								180		270	275	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*2422												
*3014L					7							
*3601, 3602		2										12
*3603		2		4					12			
*3604	1	2										12
*8001					7				w			
HLA-A allele ⁴												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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*01 subtyping.

In addition, wells number 3, 7, 10 to 12 and 20 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.

²The nucleotide position, in the 1st, 2nd or 3rd exon or the 2nd intron, 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 exon, 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.

Lot No.: **94E**

Lot-specific Information

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205	120	235	180	210	135	170	65	255	600	155	215	Length of spec. PCR product
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
			235				425			175		*2422
						19				23		*3014L
		15			18							*3601, 3602
					18							*3603
					18							*3604
												*8001
												HLA-A allele ⁴
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

⁴The nucleotide sequence of the A*0105N allele has been shown to be identical to A*0104N.

⁵Primer mix 8: Specific PCR fragment of 110 bp in the A*0107 and A*0123 alleles. Specific PCR fragment of 180 bp in the A*01010102N allele.

Primer mix 10: Specific PCR fragment of 155 bp in the A*0110 allele. Specific PCR fragment of 270 bp in the A*0129 allele.

Primer mix 11: Specific PCR fragment of 135 bp in the A*0128 allele. Specific PCR fragment of 275 bp in the A*0111N and A*0113 alleles.

Primer mix 12 might faintly amplify the A*0227, A*110101-110202, 1104-1107, 1109-1113, 1115-1119 and 1121N-1132 alleles.

Primer mix 16: Specific PCR fragment of 180 bp in the A*01010102N allele. Specific PCR fragment of 235 bp in the A*0115N allele.

Primer mix 20: Specific PCR fragment of 65 bp in the A*0110, A*0121 and A*0126 alleles. Specific PCR fragment of 425 bp in the A*0119 allele.

Primer mix 23: Specific PCR fragment of 155 bp in the A*0124 alleles. Specific PCR fragment of 175 bp in the A*0130 and *2422 alleles.

'w', might be faintly amplified.

CELL LINE VALIDATION SHEET																				
HLA-A*01 SSP subtyping kit																				
				Well																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Prod. No.:	200622901	200735502	200622903	200622904	200622905	200735506	200622907	200849608	200735509	200849610	200849611	200622912	200735513	200622914	200622915	200735516
	IHWC cell line		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	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	9366 DAUDI		*0102	*6601	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-

CELL LINE VALIDATION SHEET												
HLA-A*01 SSP subtyping kit												
				Well								
				17	18	19	20	21	22	23	24	
				Prod. No.:	200622917	200622918	200849619	200735520	200735521	200735522	200849623	200735524
IHWC cell line			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	-	-	-	-	-	-	-	+
49	9366	DAUDI	*0102	*6601	-	+	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-A*01 SSP

Product number: 101.411-24/06 – including *Taq* pol.
Lot number: 94E
Expiry date: 2010-August-01
Number of tests: 24 test – Product No. 101.411-24
6 tests – Product No. 101.411-06
Number of wells per test: 24

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2006-229-01	9	2007-355-09	17	2006-229-17
2	2007-355-02	10	2008-496-10	18	2006-229-18
3	2006-229-03	11	2008-496-11	19	2008-496-19
4	2006-229-04	12	2006-229-12	20	2007-355-20
5	2007-355-05	13	2007-355-13	21	2007-355-21
6	2006-229-06	14	2006-229-14	22	2007-355-22
7	2006-229-07	15	2006-229-15	23	2008-496-23
8	2008-496-08	16	2007-355-16	24	2007-355-24

The specificity of each primer solution of the kit has been tested against 49 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 6, 8, 9 to 11, 14, 15, 17, 19 to 23 were available. The specificities of primers in primer solutions 8, 10, 11, 14, 19 to 21 and 23 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 6 and it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solutions 9, 17 and 22 it was only possible to test the 5'-primers, the 3'-primers 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

Declaration of Conformity

Product name: *Olerup* SSP® HLA-A*01
Product number: 101.411-24/06
Lot number: 94E

Intended use: HLA-A*01 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, 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

Lot No.: **94E**

Lot-specific Information

www.olerup.com

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