

# Olerup SSP AB

## Olerup SSP™ HLA-A\*01

**Prod. No:** 101.411-24/06 – including *Taq* pol.  
101.411-24u/06u – without *Taq* pol.

**Lot No:** X98

**Expiry Date:** 2009-June-01

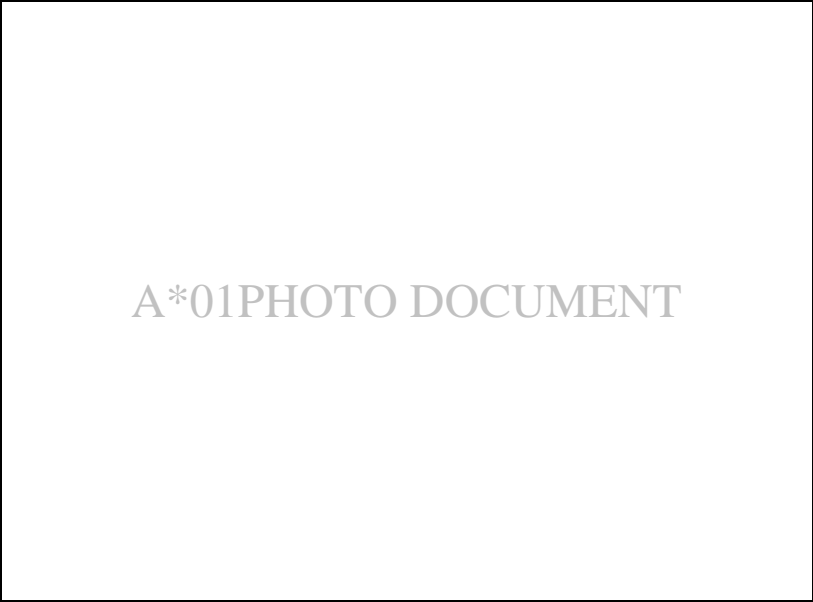
**Name:** \_\_\_\_\_ **Sample ID:** \_\_\_\_\_

**DNA Extract Date:** \_\_\_\_\_ **Conc.(ug/ul):** \_\_\_\_\_

**Test Date:** \_\_\_\_\_ **Review Date:** \_\_\_\_\_

**Tested By:** \_\_\_\_\_ **Reviewed By:** \_\_\_\_\_

**Interpretation:** \_\_\_\_\_



### Specificities and sizes of the PCR products of the 24 primer mixes used for HLA-A\*01 SSP typing.

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-A*01 alleles	Other amplified HLA-A alleles <sup>3</sup>
1	235 bp	800 bp	01010101-0102, 0104N, 0106, 0107, 0109-0111N, 0113, 0115N, 0116N-0118N, 0121-0127N	0318, 110101-1103, 1105-1125, 1128-1132, 3604
2 <sup>4</sup>	145 bp	1070 bp	01010101-010104, 0103, 0104N, 0106-0119, 0121-0127N	3601-3604
3 <sup>4</sup>	120 bp	800 bp	0102, 0120	
4	305 bp	1070 bp	0103	1126, 2632, 3313, 3603, 7410
5 <sup>5</sup>	470 bp	1070 bp	0104N	0321N, 1121N, 2307N, 2411N
6	210 bp	1070 bp	0109	
7 <sup>6</sup>	215 bp	800 bp	0106	0305, 1124, 1125, 1131, 300101-3003, 3007-3016, 3018-3022, 3103, 3104, 3402-3404, 3407, 3408, 8001
8 <sup>4</sup>	110 bp	1070 bp	0107, 0123	
9	235 bp	1070 bp	0108, 0127N	
10	155 bp	800 bp	0110	1114 <sup>weakly</sup> , 8001 <sup>weakly</sup>
11 <sup>4</sup>	275 bp	800 bp	0111N	
12 <sup>4,7</sup>	85 bp	800 bp		3601-3604
13	205 bp	1070 bp	0112, 0119, 0121	0302, 0307 <sup>weakly</sup> , 0310, 1131 <sup>weakly</sup> , 2451, 3004 <sup>weakly</sup> , 3006 <sup>weakly</sup> , 3009 <sup>weakly</sup> , 3017 <sup>weakly</sup> , 3103 <sup>weakly</sup> , 3104 <sup>weakly</sup>
14 <sup>4</sup>	120 bp	1070 bp	0113, 0117	
15 <sup>5</sup>	235 bp	1070 bp	0112, 0114, 0119	03010101-0317, 0319-0330, 1104, 1127, 2451, 300101-3004, 3006-3013, 3015-3022, 3103, 3104, 3204, 3402-3404, 3407, 3408, 3601, 3602
16 <sup>8</sup>	180, 235 bp	1070 bp	01010102N, 0115N	
17	210 bp	1070 bp	0116N	
18 <sup>4</sup>	135 bp	1070 bp	01010101-0104N, 0106, 0108-0112, 0114-0127N	3601-3604
19	165 bp	1070 bp	0118N	

<b>20<sup>4,9</sup></b>	60, 425 bp	<b>800 bp</b>	0110, 0119, 0121, 0126	
<b>21</b>	255 bp	1070 bp	0120	0219, 0236, 0237, 0254, 2414
<b>22</b>	600 bp	1070 bp	0122N	
<b>23</b>	155 bp	1070 bp	0124	
<b>24<sup>5</sup></b>	215 bp	1070 bp	0112, 0119, 0125	0302, 0310, 110101-1107, 1109-1122, 1127-1130, 1132, 2451

<sup>1</sup> 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 subtypings.

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 bp or more. Size differences shorter than 20 bp are not given. For high resolution SSP kits the 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 band may sometimes be observed. Such bands can 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.

<sup>2</sup> 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 tubes, or a band of 800 base pairs, for some tubes.

Tube 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, tubes 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.

<sup>3</sup> 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, 21 and 24.

<sup>4</sup> Short specific PCR fragments are less intense and not as sharp as longer specific bands.

<sup>5</sup> Primer mixes 5, 15 and 24 may give rise to non-specific amplifications.

<sup>6</sup> Primer mix 7 may give rise to primer oligomer formation.

<sup>7</sup> Primer mix 12 might faintly amplify the A\*0227, A\*110101-110202, 1104-1107, 1109-1113, 1115-1119 and 1121N-1132 alleles.

<sup>8</sup> 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.

The amplification patterns of the A\*01010102N and A\*0115N alleles only differ by the different lengths of the specific PCR products generated by primer mix 16.

<sup>9</sup> Primer mix 20: Specific PCR fragment of 60 bp in the A\*0110, A\*0121 and A\*0126 alleles. Specific PCR fragment of 425 bp in the A\*0119 allele.

Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
<b>HLA-A allele<sup>1</sup></b>																								
<b>*01010101, 010102-010104</b>	1	2																18						
<b>*01010102N</b>	1	2														16		18						
<b>*0102</b>	1		3															18						
<b>*0103</b>		2		4														18						
<b>*0104N</b>	1	2			5													18						
<b>*0106</b>	1	2					7											18						
<b>*0107</b>	1	2						8																
<b>*0108</b>		2							9									18						
<b>*0109</b>	1	2				6												18						
<b>*0110</b>	1	2							10									18		20				
<b>*0111N</b>	1	2								11								18						
<b>*0112</b>		2											13		15			18						24
<b>*0113</b>	1	2												14										
<b>*0114</b>		2													15			18						
<b>*0115N</b>	1	2														16		18						
<b>*0116N</b>	1	2															17	18						
<b>*0117</b>	1	2												14				18						
<b>*0118N</b>	1	2																18	19					
<b>*0119</b>		2											13		15			18		20				24
<b>*0120</b>			3															18			21			

*0121	1	2										13				18		20								
*0122N	1	2														18							22			
*0123	1	2						8								18										
*0124	1	2														18								23		
*0125	1	2														18									24	
*0126	1	2														18		20								
*0127N	1	2										9				18										
*0219, 0236, 0237, 0254, 2414																								21		
*03010101-030105, 0303N, 0304, 0306, 0308, 0309, 0311N-0317, 0319, 0320, 0322-0330, 3204															15											
*0302, 0310, 2451													13	15											24	
*0305, 300101-3003, 3007, 3008, 3010-3013, 3015, 3016, 3018-3022, 3402-3404, 3407, 3408								7							15											
*0307, 3004, 3006, 3017													w	15												
*0318, 1108, 1123	1																									
*0321N								5								15										
*110101-1103, 1105-1107, 1109-1113, 1115-1120, 1122, 1128-1130, 1132	1																								24	
*1104, 1127															15										24	
*1114	1										w														24	
*1121N	1							5																	24	
*1124, 1125	1								7																	
*1126, 2632, 3313, 7410								4																		
*1131	1								7				w													
*2307N, 2411N								5																		
*3009, 3103, 3104									7				w	15												
*3014L									7																	
*3601, 3602		2													12		15									
*3603		2		4											12											
*3604	1	2													12											
*8001								7				w														
Length of spec. PCR product(s) <sup>2</sup>	235	145	120	305	470	210	215	110	235	155	275	85	205	120	235	180	210	135	165	65	255	600	155	215		
																235	180			425						
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
Specific Reaction																										
1st match: A*																										
2nd match: A*																										
Control Reaction																										
Failed Controls																										
False Positive																										
False Negative																										

<sup>1</sup>The nucleotide sequence of the A\*0105N allele has been shown to be identical to A\*0104N.

The nucleotide sequence of the A\*0128 allele is not yet retrievable.

The amplification patterns of the A\*01010102N and A\*0115N alleles only differ by the different lengths of the specific PCR products generated by primer mix 16.

<sup>2</sup>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 60 bp in the A\*0110, A\*0121 and A\*0126 alleles. Specific PCR fragment of 425 bp in the A\*0119 allele.

Changes in revision R01 compared to R00:

1. The A\*0115N allele will be amplified by primer mix 1. Corrected in the Specificity and Interpretation Tables.

Changes in revision R02 compared to R01:

2. Footnote 1 of the Specificity Table has been expanded.