

Olerup SSP® HLA-A*32

Product number:	101.431-12 – including <i>Taq</i> polymerase
Lot number:	61K
Expiry date:	2013-May-01
Number of tests:	12
Number of wells per test:	20
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. 61K.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*® HLA-A*32 LOT

The HLA-A*32 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP*® HLA-A*32 lot was made (Lot No. 48G).

Four wells have been added to the HLA-A*32 kit,
wells **17 to 20**.

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

Well	5'-primer	3'-primer	rationale
17	New	New	New primer pair for the A*32:21 allele.
18	New	New	New primer pair for the A*32:24 allele.
19	New	New	New primer pair for the A*32:25 allele.
20	New	New	New primer pair for the A*32:23 allele.

PRODUCT DESCRIPTION

HLA-A*32 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*32:01 to A*32:25 alleles.

PLATE LAYOUT

Each test consists of 20 PCR reactions in a 24 well cut PCR plate. Wells 21 to 24 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	empty	empty	empty	empty

The 24 well cut PCR plate is marked with 'HLA-A*32' in silver/gray ink.

Well No. 1 is marked with the Lot No. '61K'.

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 covered with a PCR-compatible foil.

Please note: When removing each 24 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*32 SSP subtypings will be influenced by most A*01, eleven A*02, most A*03, most A*23, five A*24, the A*25:03, two A*26, four A*29, most A*30, seven A*31, two A*33, the A*34:08, the A*36, A*68:45 and most A*74 alleles when present on the other haplotype.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*32 alleles, i.e. **A*32:01 to A*32:25 alleles**, recognized by the HLA Nomenclature Committee in July 2010¹ will give rise to unique amplification patterns by the primers in the HLA-A*32 subtyping kit.

The A*32 subtyping kit cannot distinguish the A*32:01:01 to A*32:01:05 alleles.

¹HLA-A alleles listed on the IMGT/HLA web page 2010-July-16, release 3.1.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 35 alleles generate 25 amplification patterns that can be combined in 325 homozygous and heterozygous combinations. 165 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.

++++-----	+-----	----	*32:02, *32:07 = *32:02, *32:19N
++++-----	+-----	----	*32:02, *32:11Q = *32:02, *32:15
++++-----	+-----	----	*32:01:01, *32:02 = *32:02, *32:06 = *32:06, *32:22
++++-----	+-----	----	*32:07, *32:22 = *32:19N, *32:22
++++-----	+-----	----	*32:11Q, *32:22 = *32:15, *32:22
++++-----	+-----	----	*32:06, *32:07 = *32:06, *32:19N
++++-----	+-----	----	*32:06, *32:10 = *32:06, *32:16
++++-----	+-----	----	*32:06, *32:11Q = *32:06, *32:15
++++-----	+-----	----	*32:01:01, *32:06 = *32:06, *32:06
++++-----	+-----	----	*32:03, *32:07 = *32:03, *32:19N
++++-----	+-----	----	*32:03, *32:10 = *32:03, *32:16
++++-----	+-----	----	*32:03, *32:11Q = *32:03, *32:15
++++-----	+-----	----	*32:01:01, *32:03 = *32:03, *32:03
++++-----	+-----	----	*32:07, *32:20 = *32:19N, *32:20
++++-----	+-----	----	*32:04, *32:07 = *32:04, *32:19N
++++-----	+-----	----	*32:10, *32:20 = *32:16, *32:20
++++-----	+-----	----	*32:11Q, *32:20 = *32:15, *32:20
++++-----	+-----	----	*32:01:01, *32:20 = *32:04, *32:09 = *32:04, *32:20 = *32:09, *32:20 = *32:20, *32:20
++++-----	+-----	----	*32:04, *32:10 = *32:04, *32:16
++++-----	+-----	----	*32:04, *32:11Q = *32:04, *32:15
++++-----	+-----	----	*32:07, *32:17 = *32:17, *32:19N
++++-----	+-----	----	*32:05, *32:07 = *32:05, *32:19N
++++-----	+-----	----	*32:10, *32:17 = *32:16, *32:17
++++-----	+-----	----	*32:11Q, *32:17 = *32:15, *32:17
++++-----	+-----	----	*32:01:01, *32:17 = *32:05, *32:08 = *32:05, *32:17 = *32:08, *32:17 = *32:17, *32:17
++++-----	+-----	----	*32:05, *32:10 = *32:05, *32:16
++++-----	+-----	----	*32:05, *32:11Q = *32:05, *32:15
++++-----	+-----	----	*32:01:01, *32:05 = *32:05, *32:05
++++-----	+-----	----	*32:07, *32:08 = *32:08, *32:19N
++++-----	+-----	----	*32:07, *32:09 = *32:09, *32:19N
++++-----	+-----	----	*32:07, *32:10 = *32:07, *32:16 = *32:10, *32:19N = *32:16, *32:19N
++++-----	+-----	----	*32:07, *32:11Q = *32:11Q, *32:19N = *32:15, *32:19N
++++-----	+-----	----	*32:07, *32:18 = *32:18, *32:19N
++++-----	+-----	----	*32:07, *32:12 = *32:12, *32:19N
++++-----	+-----	----	*32:07, *32:13 = *32:13, *32:19N
++++-----	+-----	----	*32:07, *32:14 = *32:14, *32:19N
++++-----	+-----	----	*32:07, *32:21 = *32:19N, *32:21
++++-----	+-----	----	*32:07, *32:24 = *32:19N, *32:24
++++-----	+-----	----	*32:07, *32:23 = *32:19N, *32:23

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++-----+	+-----	----	*32:01:01, *32:07 = *32:01:01, *32:19N = *32:07, *32:19N = *32:19N, *32:19N
++-----	++-+-----	----	*32:08, *32:10 = *32:08, *32:16
++-----	++-+-----	----	*32:08, *32:11Q = *32:08, *32:15
++-----	++-----	----	*32:01:01, *32:08 = *32:08, *32:08
++-----	++-+-----	----	*32:09, *32:10 = *32:09, *32:16
++-----	++-+-----	----	*32:09, *32:11Q = *32:09, *32:15
++-----	++-+-----	----	*32:01:01, *32:09 = *32:09, *32:09
++-----	++-+-----	----	*32:10, *32:11Q = *32:10, *32:15 = *32:11Q, *32:16 = *32:15, *32:16
++-----	++-+-----	----	*32:10, *32:18 = *32:16, *32:18
++-----	++-+-----	----	*32:10, *32:12 = *32:12, *32:16
++-----	++-+-----	----	*32:10, *32:13 = *32:13, *32:16
++-----	++-+-----	----	*32:10, *32:14 = *32:14, *32:16
++-----	++-+-----	++--	*32:10, *32:21 = *32:16, *32:21
++-----	++-+-----	-+--	*32:10, *32:24 = *32:16, *32:24
++-----	++-+-----	--+-	*32:10, *32:25 = *32:16, *32:25
++-----	++-+-----	----+	*32:10, *32:23 = *32:16, *32:23
++-----	++-+-----	----	*32:01:01, *32:10 = *32:01:01, *32:16 = *32:10, *32:16 = *32:16, *32:16
++-----	++-+-----	----	*32:11Q, *32:18 = *32:15, *32:18
++-----	++-+-----	----	*32:11Q, *32:12 = *32:12, *32:15
++-----	++-+-----	----	*32:11Q, *32:13 = *32:13, *32:15
++-----	++-+-----	----	*32:11Q, *32:14 = *32:14, *32:15
++-----	++-+-----	++--	*32:11Q, *32:21 = *32:15, *32:21
++-----	++-+-----	-+--	*32:11Q, *32:24 = *32:15, *32:24
++-----	++-+-----	----+	*32:11Q, *32:23 = *32:15, *32:23
++-----	++-+-----	----	*32:01:01, *32:11Q = *32:01:01, *32:15 = *32:11Q, *32:11Q = *32:11Q, *32:15
++-----	++-+-----	----	*32:01:01, *32:18 = *32:12, *32:13 = *32:12, *32:18 = *32:13, *32:18 = *32:18, *32:18
++-----	++-+-----	----	*32:01:01, *32:12 = *32:12, *32:12
++-----	++-+-----	----	*32:01:01, *32:13 = *32:13, *32:13
++-----	++-+-----	----	*32:01:01, *32:14 = *32:14, *32:14
++-----	++-+-----	++--	*32:01:01, *32:21 = *32:21, *32:21
++-----	++-+-----	-+--	*32:01:01, *32:24 = *32:24, *32:24
++-----	++-+-----	----+	*32:01:01, *32:23 = *32:23, *32:23
++-+-----	++-+-----	----	*32:02, *32:02 = *32:02, *32:22

*32:01:01 = *32:01:01-32:01:05

SPECIFICITY TABLE

HLA-A*32 SSP subtyping

Specificities and sizes of the PCR products of the 20 primer mixes used for HLA-A*32 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*32 alleles	Other amplified HLA-A alleles ³
1	200 bp	800 bp	*32:01:01-32:06, 32:08-32:14, 32:16-32:24	*01:01:01:01-01:01:19, 01:03-01:04N, 01:06- 01:19, 01:21-01:42, 01:44-01:46, 01:48- 01:50, 01:52N-01:59, 01:61-01:66, 02:45, 02:56:01-02:56:02, 02:103, 02:195, 03:01:01:01-03:01:03, 03:01:05-03:11N, 03:13- 03:22, 03:25-03:29, 03:31-03:35, 03:37- 03:58, 03:60-03:69N, 03:71, 03:73-03:82, 31:21, 36:01-36:05, 74:01-74:03, 74:05- 74:14N
2	510 bp	1070 bp	*32:01:01- 32:01:05, 32:03- 32:09, 32:11Q- 32:21, 32:23- 32:25	*02:81, 02:87, 02:112, 02:124, 02:129, 23:01:01- 23:01:02, 23:03:01-23:13, 23:15-23:26, 24:13:01, 24:18, 24:24, 24:94, 29:13, 31:07-31:08, 31:10
3⁴	130 bp	1070 bp	*32:02, 32:22	*29:05, 31:24
4^{4,5}	115 bp	800 bp	*32:02, 32:06	
5	165 bp	800 bp	*32:03	
6^{4,7}	120 bp, 520 bp	1070 bp	*32:04, 32:20	*24:18
7⁴	135 bp	800 bp	*32:05, 32:17	*29:13
8^{6,8}	165 bp, 215 bp	1070 bp	*32:07, 32:19N	*01:02 ^w , 01:20 ^w , 03:72, 23:09 ^w , 24:24, 24:67, 26:16, 30:01:01-30:04, 30:06, 30:09-30:20, 30:23-30:30, 30:32- 30:41, 68:45

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9	180 bp	1070 bp	*32:01:01-32:03, 32:05-32:25	*29:13
10	150 bp	800 bp	*32:08, 32:17	*29:13
11^{4,9}	120 bp, 165 bp	1070 bp	*32:09, 32:20	
12^{4,10}	130 bp, 195 bp	1070 bp	*32:10, 32:16	*31:30
13¹¹	150 bp, 200 bp	1070 bp	*32:11Q, 32:15	*01:51, 02:55, 03:24, 25:03, 26:20, 34:08
14	215 bp	1070 bp	*32:12, 32:18	*29:06
15¹²	165 bp, 220 bp	1070 bp	*32:13, 32:18	*23:03:01, 29:03, 31:05, 33:10
16	220 bp	1070 bp	*32:14	
17⁴	75 bp	1070 bp	*32:21	*33:16
18⁴	130 bp	1070 bp	*32:24	
19⁴	120 bp	1070 bp	*32:25	
20^{4,5}	125 bp	1070 bp	*32:23	

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*32 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.

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*01 subtyping.

In addition, wells number 4, 5, 7 and 10 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*32 alleles will be amplified by primer mixes 1 to 3, 6 to 10, 12 to 15 and 17.

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

⁵Primer mixes 4 and 20 have a tendency of giving rise to non-specific amplifications.

⁶Primer mix 8 gives a lower yield of specific product than the other HLA-A*32 primer mixes.

⁷Primer mix 6: Specific PCR fragment of 120 bp in A*32:20 allele. Specific PCR fragment of 520 bp in the A*32:04 and the A*24:18 alleles.

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⁸Primer mix 8: Specific PCR fragment of 165 bp in A*32:19N allele. Specific PCR fragment of 215 bp in the A*32:07 and the A*01:02^w, 01:20^w, 03:72, 23:09^w, 24:24, 24:67, 26:16, 30:01:01-30:04, 30:06, 30:09-30:20, 30:23-30:30, 30:32-30:41 and 68:45 alleles.

⁹Primer mix 11: Specific PCR fragment of 120 bp in A*32:20 allele. Specific PCR fragment of 165 bp in the A*32:09 allele.

¹⁰Primer mix 12: Specific PCR fragment of 130 bp in A*32:10 and in the A*31:30 alleles. Specific PCR fragment of 195 bp in the A*32:16 allele.

¹¹Primer mix 13: Specific PCR fragment of 150 bp in A*32:11Q allele. Specific PCR fragment of 200 bp in the A*32:15 and in the A*01:51, 02:55, 03:24, 25:03, 26:20 and 34:08 alleles.

¹²Primer mix 15: Specific PCR fragment of 165 bp in A*32:13 and the A*23:03:01, 29:03, 31:05, 33:10 alleles. Specific PCR fragment of 220 bp in the A*32:18 allele.

‘w’, might be weakly amplified.

INTERPRETATION TABLE												
HLA-A*32 SSP subtyping												
Amplification patterns of the A*32:01 to A*32:25 alleles												
	Well ⁴											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	200	510	130	115	165	120	135	165	180	150	120	130
PCR product(s)						520		215			165	195
Length of int.	800	1070	1070	800	800	1070	800	1070	1070	800	1070	1070
pos. control ¹												
5'-primer(s) ²	98	317	448	448	180	101	180	98	180	180	101	448
	5'-CTT 3'	5'-gCT 3'	5'-CCT 3'	5'-CCT 3'	5'-TTT 3'	5'-CAT 3'	5'-TTT 3'	5'-CTC 3'	5'-TTT 3'	5'-TTT 3'	5'-CAT 3'	5'-CCT 3'
						317		448			448	
						5'-gCT 3'		5'-CCT 3'			5'-CCT 3'	
3'-primer(s) ³	256	538	539	524	302	180	265	270	317	282	180	539
	5'-CTg 3'	5'-CAA 3'	5'-TCT 3'	5'-CAT 3'	5'-ggT 3'	5'-TCA 3'	5'-CCC 3'	5'-ACA 3'	5'-ggA 3'	5'-gAC 3'	5'-TCA 3'	5'-TCC 3'
	256	555				555	282	573		292	571	602
	5'-CTg 3'	5'-CCA 3'				5'-CCA 3'	5'-gAC 3'	5'-AgT 3'		5'-gTg 3'	5'-CCg 3'	5'-TCA 3'
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-A allele												
*32:01:01-32:01:05	1	2							9			
*32:02	1		3	4					9			
*32:03	1	2			5				9			
*32:04	1	2				6						
*32:05	1	2					7		9			
*32:06	1	2		4					9			
*32:07		2						8	9			
*32:08	1	2							9	10		
*32:09	1	2							9		11	
*32:10	1								9			12
*32:11Q	1	2							9			
*32:12	1	2							9			
*32:13	1	2							9			
*32:14	1	2							9			
*32:15		2							9			
*32:16	1	2							9			12
*32:17	1	2					7		9	10		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE								
HLA-A*32 SSP subtyping								
Amplification patterns of the A*32:01 to A*32:25 alleles								
Well⁴								
13	14	15	16	17	18	19	20	
150	215	165	220	75	130	120	125	Length of spec. PCR product(s)
200		220						
1070	1070	1070	1070	1070	1070	1070	1070	Length of int. pos. control ¹
102	139	139	124	448	180	180	448	5'-primer(s) ²
5'-ACA 3'	5'-TCg 3'	5'-TCg 3'	5'-gCC 3'	5'-CCT 3'	5'-TTT 3'	5'-TTT 3'	5'-CCT 3'	
448	448	448						
5'-CCT 3'	5'-CCT 3'	5'-CCT 3'						
259	317	317	302	482	271	258	532	3'-primer(s) ³
5'-gTT 3'	5'-ggA 3'	5'-ggA 3'	5'-ggC 3'	5'-Tgg 3'	5'-CAT 3'	5'-TCg 3'	5'-CTT 3'	
559	616	570						
5'-CTC 3'	5'-CgC 3'	5'-CCg 3'						
13	14	15	16	17	18	19	20	Well No.
								HLA-A allele
								*32:01:01-32:01:05
								*32:02
								*32:03
								*32:04
								*32:05
								*32:06
								*32:07
								*32:08
								*32:09
								*32:10
13								*32:11Q
	14							*32:12
		15						*32:13
			16					*32:14
13								*32:15
								*32:16
								*32:17
13	14	15	16	17	18	19	20	Well No.

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Length of spec.	200	510	130	115	165	120	135	165	180	150	120	130
PCR product(s)						520		215			165	195
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*32:18	1	2							9			
*32:19N	1	2						8	9			
*32:20	1	2				6			9		11	
*32:21	1	2							9			
*32:22	1		3						9			
*32:23	1	2							9			
*32:24	1	2							9			
*32:25		2							9			
*01:01:01:01-01:01:19, 01:03-01:04N, 01:06-01:19, 01:21-01:42, 01:44-01:46, 01:48-01:50, 01:52N-01:59, 01:61-01:66, 02:45, 02:56:01-02:56:02, 02:103, 02:195, 03:01:01:01-03:01:03, 03:01:05-03:11N, 03:13-03:22, 03:25-03:29, 03:31-03:35, 03:37-03:58, 03:60-03:69N, 03:71, 03:73-03:82, 31:21, 36:01-36:05, 74:01-74:03, 74:05-74:14N	1											
*01:02, 01:20								w				
*01:51, 02:55, 03:24, 25:03, 26:20, 34:08												
*02:81, 02:87, 02:112, 02:124, 02:129, 23:01:01-23:01:02, 23:03:02-23:08N, 23:10-23:13, 23:15-23:26, 24:13:01, 24:94, 31:07-31:08, 31:10		2										
*03:72, 24:67, 26:16, 30:01:01-30:04, 30:06, 30:09-30:20, 30:23-30:30, 30:32-30:41, 68:45								8				
*23:03:01		2										
*23:09		2						w				
*24:18		2				6						
*24:24		2						8				
*29:03, 31:05, 33:10												
*29:05, 31:24			3									
*29:06												
*29:13		2					7		9	10		
*31:30												12
*33:16												
HLA-A allele												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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150	215	165	220	75	130	120	125	Length of spec. PCR product(s)
200		220						
13	14	15	16	17	18	19	20	Well No.
	14	15						*32:18
								*32:19N
								*32:20
				17				*32:21
								*32:22
							20	*32:23
					18			*32:24
						19		*32:25
								*01:01:01:01-01:01:19, 01:03-01:04N, 01:06-01:19, 01:21-01:42, 01:44-01:46, 01:48-01:50, 01:52N-01:59, 01:61-01:66, 02:45, 02:56:01-02:56:02, 02:103, 02:195, 03:01:01:01-03:01:03, 03:01:05-03:11N, 03:13-03:22, 03:25-03:29, 03:31-03:35, 03:37-03:58, 03:60-03:69N, 03:71, 03:73-03:82, 31:21, 36:01-36:05, 74:01-74:03, 74:05-74:14N
								*01:02, 01:20
13								*01:51, 02:55, 03:24, 25:03, 26:20, 34:08
								*02:81, 02:87, 02:112, 02:124, 02:129, 23:01:01-23:01:02, 23:03:02-23:08N, 23:10-23:13, 23:15-23:26, 24:13:01, 24:94, 31:07-31:08, 31:10
								*03:72, 24:67, 26:16, 30:01:01-30:04, 30:06, 30:09-30:20, 30:23-30:30, 30:32-30:41, 68:45
		15						*23:03:01
								*23:09
								*24:18
								*24:24
		15						*29:03, 31:05, 33:10
								*29:05, 31:24
	14							*29:06
								*29:13
								*31:30
				17				*33:16
								HLA-A allele
13	14	15	16	17	18	19	20	Well No.

Lot No.: **61K**

Lot-specific Information

www.olerup-ssp.com

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*32 subtyping.

In addition, wells number 4, 5, 7 and 10 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 2nd or 3rd exon, 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.

⁴Primer mix 6: Specific PCR fragment of 120 bp in A*32:20 allele. Specific PCR fragment of 520 bp in the A*32:04 and the A*24:18 alleles.

Primer mix 8: Specific PCR fragment of 165 bp in A*32:19N allele. Specific PCR fragment of 215 bp in the A*32:07 and the A*01:02^w, 01:20^w, 03:72, 23:09^w, 24:24, 24:67, 26:16, 30:01:01-30:04, 30:06, 30:09-30:20, 30:23-30:30, 30:32-30:41 and 68:45 alleles.

Primer mix 11: Specific PCR fragment of 120 bp in A*32:20 allele. Specific PCR fragment of 165 bp in the A*32:09 allele.

Primer mix 12: Specific PCR fragment of 130 bp in A*32:10 and in the A*31:30 alleles. Specific PCR fragment of 195 bp in the A*32:16 allele.

Primer mix 13: Specific PCR fragment of 150 bp in A*32:11Q allele. Specific PCR fragment of 200 bp in the A*32:15 and in the A*01:51, 02:55, 03:24, 25:03, 26:20 and 34:08 alleles.

Primer mix 15: Specific PCR fragment of 165 bp in A*32:13 and the A*23:03:01, 29:03, 31:05, 33:10 alleles. Specific PCR fragment of 220 bp in the A*32:18 allele.

'w', might be weakly amplified.

CELL LINE VALIDATION SHEET																				
HLA-A*32 SSP subtyping kit																				
				Well																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Lot No.:	200965601	200850302	200850303	200850304	200965605	200965606	200965607	201078208	200850309	200965610	200965611	200850312	200850313	200965614	200965615	201078216
	IHWC cell line	A*	A*																	
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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Lot No.: **61K**

Lot-specific Information

www.olerup-ssp.com

CELL LINE VALIDATION SHEET									
HLA-A*32 SSP subtyping kit									
					Well				
					17	18	19	20	
					Lot No.:	201078217	201078218	201078219	201078220
	IHC cell line	A*	A*						
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*32 SSP

Product number: 101.431-12 – including *Taq* polymerase
Lot number: 61K
Expiry date: 2013-May-01
Number of tests: 12
Number of wells per test: 20

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2009-656-01	9	2008-503-09	17	2010-782-17
2	2008-503-02	10	2009-656-10	18	2010-782-18
3	2008-503-03	11	2009-656-11	19	2010-782-19
4	2008-503-04	12	2008-503-12	20	2010-782-20
5	2009-656-05	13	2008-503-13		
6	2009-656-06	14	2009-656-14		
7	2009-656-07	15	2009-656-15		
8	2010-782-08	16	2010-782-16		

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 3 to 5, 7 and 10 to 20 were available. The specificities of the primers in primer solutions 3 to 5, 7, 10 to 15 and 18 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer.

In primer solution 16 it was only possible to test the 3'-primer, the 5'-primer was not possible to test, and in primer solutions 17, 19 and 20 it was only possible to test the 5'-primer, the 3'-primer was not possible to test.

In primer solution 1, 8, 10, 12 and 13 one of the 3'-primers was not possible to test. In primer solutions 6 and 15 one of the 5'-primers was not possible to test, and in primer solutions 11 and 14 one 5'-primer and one 3'-primer were not possible to test. Additional primers in primer solutions 2, 6 and 8 were tested by separately adding one 5' –primer, respectively one 3'-primer.

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

Date of approval: 2010-November-11

Approved by:

Quality Control, Supervisor

Lot No.: **61K**

Lot-specific Information

www.olerup-ssp.com

Declaration of Conformity

Product name: *Olerup* SSP® HLA-A*32
Product number: 101.431-12
Lot number: 61K

Intended use: HLA-A*32 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 9001:2008 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, 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
2010-November-11

Olle Olerup

Lot No.: **61K**

Lot-specific Information

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