Olerup SSP® HLA-A low resolution

Product number:	**101.401-48u/12u – without *Taq* polymerase**
Lot number:	**62G**
Expiry date:	**2011-November-01**
Number of tests:	**48 tests – Product No. 101.401-48u**
	12 tests – Product No. 101.401-12u
Number of wells per test:	**23 +1**
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. 62G.

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

The **HLA-A low resolution** specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup* SSP® HLA-A low resolution lot was made **(Lot No. 26F)**.

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

Well	5'-primer	3'-primer	rationale
1	Added	-	Primer added for the A*0143 allele.
15	Added	Added	Primer pair added for the A*3026 and 3028 alleles.
17	Added	Added	Primer pair added for improved resolution.
18	-	Added	Primer added for the A*3321 allele.
20	Added	-	Primer added for the A*6848 allele.

Changes in revision R01 compared to R00:

1. Primer mix 3 may faintly amplify the A*30:04, 30:06, 30:17 and 30:29 alleles.

Well 24 contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup* SSP® HLA Class I, DRB, DQB1 and DPB1 amplicons as well as the amplicons generated by control primer pairs.

PCR product sizes range from 75 to 430 base pairs.
The PCR product generated by the control primer pair is 430 base pairs.

Length of PCR product	**105**	**200**	**105**	**80**	**75**	**80**
5'-primer[1]	**164**	**340**	**440**	**45**	**45**	**43**
	5'-CAC3'	**5'-Agg3'**	**5'-TTA3'**	**5'-Tg g3'**	**5'-Tg g3'**	**5'-Tg g3'**
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**
A*	**+**	**+**	**+**			
B*	**+**	**+**	**+**			
Cw*	**+**	**+**	**+**			
DRB1				**+**	**+**	
DRB3				**+**	**+**	
DRB5				**+**		
DQB1					**+**	
DPB1						**+**

[1]The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and codonnumbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.
[2]The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

PRODUCT DESCRIPTION

HLA-A low resolution

CONTENT

The primer set contains 5'- and 3'-primers for grouping the HLA-A*0101 to A*8001 alleles into the corresponding serological groups A1 to A80.

PLATE LAYOUT

Each test consists of 24 PCR reactions in a 24 well 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**

Wells 1 to 23 – HLA-A low resolution primers.
Well 24 – Negative Control.
The 24 well PCR plate is marked with 'HLA-A low' in silver/gray ink.
Well No. 1 is marked with the Lot No. '62G'.
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

Only HLA-A alleles will be amplified by the 23 wells of the HLA-A low resolution primer set except that primer mix 6 will amplify the B*1827 allele. Thus, the interpretation of HLA-A low resolution is only influenced by this HLA-B allele and not by other HLA Class I genes.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A alleles, i.e. **A*0101 to A*8001**, recognized by the HLA Nomenclature Committee in October 2009[1] will be amplified by the primers in the HLA-A low resolution primer set. The HLA-A alleles will be grouped into their corresponding serological specificities[2].

[1]HLA-A, HLA-B and HLA-Cw alleles listed on the IMGT/HLA web page 2009-October-19, release 2.27.0, www.ebi.ac.uk/imgt/hla.
[2]The A*0318 and A*2619 alleles will give rise to identical amplification patterns. These two alleles can be separated by the respective high resolution SSP primer sets.
The A*2314, A*2405, A*241302 and A*2424 alleles will give rise to identical amplification patterns. These four alleles can be separated by the respective high resolution SSP primer sets.
The A*2631 and A*6611 alleles will give rise to identical amplification patterns. These two alleles can be separated by the respective high resolution SSP primer sets.

SPECIFICITY TABLE

HLA-A low resolution primer set

Specificities and sizes of the PCR products of the 23 primer mixes used for HLA-A low resolution SSP typing

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	HLA-A serology[3]	Amplified HLA-A alleles[4,10,11]
1[5]	120 bp, 145 bp	**800 bp**	A1, A36	*01010101-0104N, 0106-0145, 3601-3604
2[6]	135 bp, 200 bp, 255 bp	1070 bp	A2, A19, A28, A203, A210	*02010101-020115, 020117-020119, 020121-022202, 0224-023501, 023503-0247, 0249-029702, 0299, 9201-9221, 9223-9228, 9230-9295
3[12]	205 bp, 235 bp	1070 bp	A1, A3, A11, A32, A34, A36	*0112, 0119, 0121, 03010101-0317, 0319-0362, 1125, 2492, 3029, 3204, 3402-3404, 3407, 3408, 3602
4	190 bp	**800 bp**	A1, A2, A3, A11, A26, A30, A36	*01010101-0104N, 0106, 0107, 0109-0111N, 0113, 0116N-0118N, 0120-0129, 0131N-0145, 0278, 9269, 0312, 0318, 110101-1127, 1129-1153, 2619, 3008, 3604
5	160 bp, 535 bp	**800 bp**	A3,A9,A23, A24, A31, A32	*0330, 230101-2322, 24020101-2411N, 241301-2415, 2417-2464, 2466-2488, 2490N-2499, 3108, 3205, 3213
6	165 bp, 200 bp	**800 bp**	A2,A9,A23, A24, A29, A80	*9222, 230101-2322, 2405, 241302, 2424, 2907, 8001, **B*1827**
7[7]	175 bp, 200 bp	1070 bp	A2, A23, A24, A26, A33	*021701[W], 021702[W], 2314, 24020101-2411N, 241301, 241302, 2417-2450, 2454-2456, 2458-2463, 2466-2491, 2493, 2495-2499, 2616, 3319, 6845
8	165 bp, 200 bp	**800 bp**	A2, A3, A10, A11, A25, A26, A28,A32, A34, A66, A68, A69	*0255, 0324, 0350, 1110, 250101-2510, 260101-2606, 2608-2615, 2617, 2618, 2620-2641, 3215, 340101-3408, 6601-6611, 680101-6848, 6901
9[5]	75 bp	**800 bp**	A25, A32	*250101-2510, 320101-3202, 3204, 3206-3220
10[5,7]	85 bp	1070 bp	A2, A10,	*9246, 260101-2602, 2604, 260701-

			A26, A33	2618, 2620, 2622-2629, 2632-2641, 3313
11[5,6,7]	80 bp, 175 bp, 500 bp	1070 bp	A1, A11, A24, A26, A31, A34, A66	*0113, 0128, 110101-1127, 1129-1153, 2419, 2444, 2603, 2606, 2621, 3103, 340101-3408, 6601, 6604-6610
12	185 bp	**800 bp**	A11, A25, A26, A31, A34, A43, A66	*1111, 2506, 2609, 3103, 3104, 340101-3408, 4301, 6602, 6603
13	175 bp, 225 bp	1070 bp	A1, A2, A3, A25, A26, A34, A43, A66, A74	*0113, 0234-023503, 025601, 025602, 0262, 9203, 9235, 03010101-0307, 0309-0311N, 0313-0331, 0333-0335, 0337-0340, 0342-0356, 0358, 0360-0362, 250101-2505, 2507-2510, 260101-260112, 2602[W], 2603, 2605-2608, 2610-2633, 2635-2641, 3408, 4301, 6601, 6604-6611, 7413
14	200 bp, 240 bp	1070 bp	A29, A33	*29010101-2920, 3313
15[5]	90 bp, 135 bp, 205 bp	1070 bp	A1, A30	*0113, 0128, 0343, 300101-3004, 3006-3020, 3022-3031
16	370 bp, 395 bp	1070 bp	A31, A32	*2914, 310102-3127, 3205
17	150 bp, 180 bp	1070 bp	A29, A32	*0343, 2913, 320101-3203, 3205-3220
18	200 bp	1070 bp	A33, A68	*330101-330103, 330301-3328, 6829
19	160 bp, 200 bp	**800 bp**	A74	*2919, 7401-7414N
20[8]	155 bp, 200 bp, 240 bp	**800 bp**	A2, A210, A25, A68	*0210, 0234-023503, 0239, 0246, 0248, 0250, 0252, 025601, 025602, 0262, 0270, 0273, 0278, 0293, 0295, 9203, 9210, 9214, 9217, 9222, 9229, 9256, 2494, 2505, 680101-6848
21	240 bp, 375 bp	**800 bp**	A2, A24, A26, A68, A69	*0255, 2482, 2622, 3322, 6609, 6829, 6901
22[5,9]	85 bp, 240 bp	**800 bp**	A2, A36	*0234-023503, 0246, 0248, 025601, 025602, 0262, 0270, 0278, 9203, 9229, 3601-3604
23[5,7]	75 bp, 160 bp	**800 bp**	A2, A26, A68, A80	*0255, 2603, 2605, 2606, 2621, 2630, 3324, 6805, 6815, 6820, 8001

[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 low resolution 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 inherit 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 low resolution typing.
In addition, wells number 4, 5, 6, 8, 9, 12, 19 to 23 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]The serological reactivity of all HLA-A alleles is not known. In this table we use the information in the HLA Dictionary 2008 on the www.ebi.ac.uk/imgt/hla web site, the information available at the www.worldmarrow.org web site, the expert-assigned serological grouping in Tissue Antigens (2009) **73**:95-170 and have also inferred the serological grouping from the naming of the sequence-defined allele.
[4]For several HLA-A alleles only partial 1st exon nucleotide sequences are available. We assume that unknown sequences are conserved within allelic groups.
Nucleotide sequence information is available for only exons 2 and 3 of many HLA Class I alleles and for only exon 2 of many HLA Class II alleles and not for other exons or for the introns of these alleles. We assume that unknown sequences in these exons and in the introns are conserved within loci and within allelic groups.
[5]Specific PCR fragments shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR bands.
[6]The primer pairs in vial 2 and 11 will in most samples give rise to two specific PCR fragments.
[7]Primer mixes 7, 10, 11 and 23 may yield less specific PCR product than the other primer mixes.
[8]Primer mix 20 may give rise to nonspecific amplifications.
[9]Primer mix 22 might faintly amplify most A*11 alleles.
[10]The A*0318 and A*2619 alleles will give rise to identical amplification patterns. These two alleles can be separated by the respective high resolution SSP primer sets.
The A*2314, A*2405, A*241302 and A*2424 alleles will give rise to identical amplification patterns. These four alleles can be separated by the respective high resolution SSP primer sets.
The A*2631 and A*6611 alleles will give rise to identical amplification patterns. These two alleles can be separated by the respective high resolution SSP primer sets.
[11]Primer mix 6 will amplify the B*1827 allele.
[12]Primer mix 3 may faintly amplify the A*30:04, 30:06, 30:17 and 30:29 alleles.
'w', might be weakly amplified.

INTERPRETATION TABLE

HLA-A low resolution SSP typing

Amplification patterns of the A*0101 to A*8001 alleles

	Well[6]																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Length of spec.	120	135	205	190	160	165	175	165	75	85	80	185	175	200	90	370	150	200	160	155	240	85	75
PCR product(s)	145	200	235		535	200	200	200			175		225	240	135	395	180		200	200	375	240	160
		255									500				205					240			
Length of int.	800	1070	1070	800	800	800	1070	800	800	1070	1070	800	1070	1070	1070	1070	1070	1070	800	800	800	800	800
pos. control[1]																							
5'-primer(s)[2]	98	78	363	98	144	106	98	98	266	257	302	103	98	98	203	41	180	98	180	78	28	78	176
	5'-CTT 3'	5'-TCT 3'	5'-ATA 3'	5'-CTA 3'	5'-gCC 3'	5'-CCA 3'	5'-CTC 3'	5'-CTA 3'	5'-ACg 3'	5'-Cgg 3'	5'-ggA 3'	5'-CCT 3'	5'-CTT 3'	5'-CAC 3'	5'-gAA 3'	5'-CTT 3'	5'-TTT 3'	5'-CAC 3'	5'-TTT 3'	5'-TCT 3'	5'-TCg 3'	5'-TCT 3'	5'-gCA 3'
	103	106		413	317	176	368	102	266	261	385	423	423		362		418			106	261	527	261
	5'-CCT 3'	5'-CCA 3'		5'-CCg 3'	5'-gCT 3'	5'-gCA 3'	5'-gTT 3'	5'-ACA 3'	5'-ACg 3'	5'-AAC 3'	5'-ggC 3'	5'-gCT 3'	5'-gCT 3'		5'-ggT 3'		5'-AgC 3'			5'-CCA 3'	5'-AAC 3'	5'-TgC 3'	5'-AAC 3'	
	123	144				368		413							363					391			
	5'-AgT 3'	5'-gCA 3'				5'-gTT 3'		5'-CCg 3'							5'-ATA 3'					5'-ACg 3'				
		144																		391			
		5'-TCA 3'																		5'-ACg 3'			
3'-primer(s)[3]	203	240	527	256	265	256	259	259	302	299	341	257	282	257	299	238	317	256	299	265	97	265	292
	5'-TCT 3'	5'-ggA 3'	5'-CCA 3'	5'-CTg 3'	5'-CCC 3'	5'-CCC 3'	5'-gTT 3'	5'-gTT 3'	5'-ggC 3'	5'-TCg 3'	5'-CgT 3'	5'-gCA 3'	5'-gAC 3'	5'-gCA 3'	5'-CCA 3'	5'-CCT 3'	5'-ggA 3'	5'-CCC 3'	5'-CCA 3'	5'-CCC 3'	5'-ggT 3'	5'-CCC 3'	5'-gTg 3'
		292	527	559	570	292	502	538			521	559	282	299	411	265	527	256	299	282	355	282	292
		5'-gTg 3'	5'-CCT 3'	5'-CCg 3'	5'-CCg 3'	5'-gTT 3'	5'-CTT 3'	5'-CCA 3'			5'-ggg 3'	5'-CTC 3'	5'-gAC 3'	5'-TCg 3'	5'-TCA 3'	5'-CCC 3'	5'-CCT 3'	5'-CTC 3'	5'-CCA 3'	5'-gAC 3'	5'-gAC 3'	5'-gAC 3'	5'-gTT 3'
			555			502	539					559	559		526			259	341	282		282	299
			5'-gCA 3'			5'-CTg 3'	5'-TCT 3'					5'-CgT 3'			5'-CCA 3'			5'-gTT 3'	5'-CgT 3'	5'-gAC 3'		5'-gAC 3'	5'-TCT 3'
			555			538														506		570	299
			5'-CCA 3'		5'-CAg 3'															5'-TgT 3'		5'-CAC 3'	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23

Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
HLA-A allele[4]	ser[5]																							
*01010101-0104N, 0106, 0107, 0109-0111N, 0116N-0118N, 0120, 0122N-0127N, 0129, 0131N-0145	A1, null	1			4																			
*0108, 0114, 0115N, 0130	A1	1																						
*0112, 0119	-	1		3																				
*0113	A1	1			4							11		13		15								
*0121	A1	1		3	4																			
*0128	-	1			4							11				15								
*02010101-020115, 020117-020119, 020121-0209, 0211-0216, 0218-022202, 0224-0233, 0236-0238, 0240-0245, 0247, 0249, 0251, 0253N, 0254, 0257-0261, 0263-0269, 0271, 0272, 027401-0277, 0279-0292, 0294N, 0296-029702, 0299, 9201, 9202, 9204-9209, 9211-9213N, 9215, 9216, 9218-9221, 9223-9228, 9230-9234, 9236-9245, 9247-9255, 9257-9268, 9270-9295	A2, A203, null		2																					
*0210, 0239, 0250, 0252, 0273, 0293, 0295, 9210, 9214, 9217, 9256	A2, A210		2																		20			
*021701, 021702	A2		2					w																
*0234, 023501, 023503, 025601, 025602, 0262, 9203	A2		2											13							20		22	
*023502	-													13							20		22	
*0246, 0270	A2		2																		20		22	
*0248, 9229	A2																				20		22	
*0255	A2/ A28		2						8													21		23
*0278	A2		2		4																20		22	
*9222	A2						6														20			
Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23

Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
*9235	-		2											13										
*9246	-		2								10													
*9269	-		2		4																			
*03010101-0307, 0309-0311N, 0313-0317, 0319-0323, 0325-0329, 0331, 0333-0335, 0337-0340, 0342, 0344-0349, 0351-0356, 0358, 0360-0362	A3, null			3										13										
*0308, 0332, 0336N, 0341, 0357, 0359	A3, null			3																				
*0312	A3			3	4																			
*0318, 2619[7]	-				4									13										
*0324, 0350	A3			3					8					13										
*0330	A3			3		5								13										
*0343	-			3										13		15		17						
*110101-1109, 1112-112402, 1126, 1127, 1129-1153	A11, null				4							11												
*1110	A11				4				8			11												
*1111	-				4							11	12											
*1125	A11			3	4							11												
*230101-2313, 2315-2322	A23					5	6																	
*2314, 2405, 241302, 2424[8]	A23, A9, A24					5	6	7																
*24020101-2404, 2406-2411N, 241301, 2417, 2418, 2420-2423, 2425-2443, 2445N-2450, 2454-2456, 2458-2463, 2466-2481, 2483N-2488, 2490N, 2491, 2493, 2495-2499	A24, A2403, null					5		7																
*2414, 2415, 2451-2453, 2457, 2464	A24					5																		
*2419, 2444	A9					5		7				11												
*2482	-					5		7														21		
*2489	-							7																
*2492	-			3		5																		
*2494	-					5															20			
Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23

Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
*250101-2504, 2507-2510	A25								8	9				13										
*2505	A25								8	9				13							20			
*2506	A25								8	9			12											
*260101-260112, 2608, 2610-2615, 2617, 2618, 2620, 2623-2629, 2632, 2633, 2635-2641	A26, A10, null								8		10			13										
*2602	A26								8		10			w										
*2603, 2606, 2621	A26								8			11		13										23
*2604, 2634	A26								8		10													
*2605, 2630	A26								8					13										23
*260701, 260702	A26										10			13										
*2609	A26								8		10		12											
*2616	A26							7			10			13										
*2622	A26								8		10			13								21		
*2631, 6611[9]	A26								8					13										
*29010101-2906, 2908N-2912, 2915-2918, 2920	A29														14									
*2907	A29						6								14									
*2913	-														14			17						
*2914	-														14		16							
*2919															14					19				
*300101-3004, 3006, 3007, 3009-3020, 3022-3028, 3030, 3031	A30															15								
*3008	A30				4											15								
*3029	-			3												15								
*310102-3102, 3105-3107, 3109-3127	A31																16							
*3103	A31											11	12				16							
*3104	A31												12				16							
*3108	A31/ A24					5											16							
*320101-3202, 3206-3212, 3214, 3216-3220	A32									9								17						
*3203	A32																	17						
*3204	A32/ A3			3						9														
*3205	A32					5											16	17						
*3213	A32					5				9								17						
*3215	A32								8	9								17						
Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23

Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
*330101-330103, 330301-3312, 3314-3318, 3320, 3321, 3323, 3325-3328	A33																		18					
*3313	-										10				14				18					
*3319	-							7											18					
*3322	-																		18			21		
*3324	-																		18					23
*340101, 340102, 3405, 3406	A34, A10								8			11	12											
*3402-3404, 3407	A34			3					8			11	12											
*3408	A34			3					8			11	12	13										
*3601, 3603	A36	1																					22	
*3602	A36	1		3																			22	
*3604	A36	1			4																		22	
*4301	A43												12	13										
*6601, 6604-6608, 6610	A66								8			11		13										
*6602, 6603	A66								8				12											
*6609	-								8			11		13								21		
*680101-6804, 6806-6814, 6816-6819, 6821-6828, 6830-6844, 6846-6848	A68, null								8												20			
*6805, 6815, 6820	A68								8												20			23
*6829	A68								8										18		20	21		
*6845	-							7	8												20			
*6901	A69								8													21		
*7401-7412N, 7414N	A74, null																			19				
*7413	-													13						19				
*8001	A80						6																	23
HLA-A allele[4]	ser[5]																							
Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
*B*1827*							6																	
Well No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23

[1]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 low resolution SSP typing.
In addition, wells number 4, 5, 6, 8, 9, 12, 19 to 23 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.
[2]The nucleotide position, in the 1st, 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.
[3]The 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.
[4]The sequence of the A*0105N has been shown to be identical to A*0104N.
The A*020116 allele has been renamed to A*9234.
The A*020120 has been renamed to A*020118.
The sequence of the A*0223 allele has been shown to be identical to A*0222.
The sequence of the A*0298 allele has been shown to be identical to A*0296.
The A*1128 allele has been renamed to A*111502.
The sequence of the A*2401 allele has been shown to be in error.
The sequence of the A*2412 allele has been shown to be identical to A*2408.
The A*2416 allele has been renamed to A*3108.
The A*2465 allele has been renamed to A*241302.
The sequence of the A*3005 allele has been shown to be identical to A*3004.
The A*3021 allele has been renamed to A*301102.
The sequence of the A*31011 allele has been shown to be identical to A*310102.
The sequence of the A*3302 allele has been shown to be identical to A*3303.
[5]The serological reactivity of all HLA-A alleles is not known. In this table we use the information in the HLA Dictionary 2008 on the www.ebi.ac.uk/imgt/hla web site, the information available at the www.worldmarrow.org web site, the expert-assigned serological grouping in Tissue Antigens (2009) **73**:95-170 and have also inferred the serological grouping from the naming of the sequence-defined allele.
[6]The primer pairs in vials 2 and 11 will in most samples give rise to two specific PCR fragments.
[7]The A*0318 and A*2619 alleles will give rise to identical amplification patterns. These two alleles can be separated by the respective high resolution SSP primer sets.
[8]The A*2314, A*2405, A*2424 and A*2465 alleles will give rise to identical amplification patterns. These two alleles can be separated by the respective high resolution SSP primer sets.
[9]The A*2631 and A*6611 alleles will give rise to identical amplification patterns. These two alleles can be separated by the respective high resolution SSP primer sets.
'w', may be weakly amplified.

CELL LINE VALIDATION SHEET

HLA-A low resolution primer set

					Well														
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Lot No.:	200966901	200855002	200855003	200855004	200855005	200855006	200855007	200855008	200855009	200855010	200855011	200855012	200959313	200855014	200855015	200855016
	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	9025	DEU	*3101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
9	9026	YAR	*2601		-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-
10	9107	LKT3	*2402		-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*2902		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
12	9052	DBB	*0201		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*0201		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*3101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*2402		-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*2902		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
17	9282	CTM3953540	*0301	*8001	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-
18	9257	32367	*3303	*7401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*0201		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*0201		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*0217		-	+	-	-	-	-	W	-	-	-	-	-	-	-	-	-
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		-	+	-	-	-	-	W	-	-	-	-	-	-	-	-	-
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	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-

CELL LINE VALIDATION SHEET

HLA-A low resolution primer set

					Well						
					17	18	19	20	21	22	23
				Lot No.:	200855017	200855018	200855019	200959320	200855021	200855022	200855023
	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	9025	DEU	*3101		-	-	-	-	-	-	-
9	9026	YAR	*2601		-	-	-	-	-	-	-
10	9107	LKT3	*2402		-	-	-	-	-	-	-
11	9051	PITOUT	*2902		-	-	-	-	-	-	-
12	9052	DBB	*0201		-	-	-	-	-	-	-
13	9004	JESTHOM	*0201		-	-	-	-	-	-	-
14	9071	OLGA	*3101		-	-	-	-	-	-	-
15	9075	DKB	*2402		-	-	-	-	-	-	-
16	9037	SWEIG007	*2902		-	-	-	-	-	-	-
17	9282	CTM3953540	*0301	*8001	-	-	-	-	-	-	+
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	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-A low resolution

Product number: **101.401-48u/12u – without *Taq* polymerase**
Lot number: **62G**
Expiry date: **2011-November-01**
Number of tests: **48 tests – Product No. 101.401-48u**
12 tests – Product No. 101.401-12u
Number of wells per test: **23 + 1**

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2009-669-01	9	2008-550-09	17	2008-550-17
2	2008-550-02	10	2008-550-10	18	2008-550-18
3	2008-550-03	11	2008-550-11	19	2008-550-19
4	2008-550-04	12	2008-550-12	20	2009-593-20
5	2008-550-05	13	2009-593-13	21	2008-550-21
6	2008-550-06	14	2008-550-14	22	2008-550-22
7	2008-550-07	15	2008-550-15	23	2008-550-23
8	2008-550-08	16	2008-550-16		

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

Additional 5'- and 3'-primers in primer solutions 4 to 8, 12, 15, 17 and 20 to 22 were tested by separately adding one 3'-primer, respectively one 5'-primer.
An additional 5'-primer in primer solution 10 were tested by separately adding one 3'-primer. Additional 3'-primers in primer solutions 3, 16, 18, 19 and 23 were tested by separately adding one 5'-primer. Two 5'-primers in primer solution 2, one 5'-primer in primer solution 15 and one 3'-primer in primer solutions 3, 18 and 19 were not possible to test.

The negative control primer pairs, **Production No. 2009-614-01**, can detect contamination with PCR products diluted 10^{-9}.

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

Date of approval: 2011-February-04

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® HLA-A low resolution
Product number: 101.401-48u/12u
Lot number: 62G

Intended use: HLA-A low 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
2011- February-04

Olle Olerup
Managing Director

ADDRESSES:

Manufacturer:
***Olerup* SSP AB**, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.
Tel: +46-8-717 88 27
Fax: +46-8-717 88 18
E-mail: info-ssp@olerup.com
Web page: http://www.olerup.com

Distributed by:
Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.
Tel: +43-1-710 15 00
Fax: +43-1-710 15 00 10
E-mail: support-at@olerup.com
Web page: http://www.olerup.com

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382
Tel: 1-877-OLERUP1
Fax: 610-344-7989
E-mail: info.us@olerup.com
Web page: http://www.olerup.com

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