

Olerup SSP™ HLA-A low resolution

Product number:	101.401-48/12 – licensed for PCR 101.401-48u/12u – <u>not</u> licensed for PCR
Lot number:	X92
Expiry date:	2009-June-01
Number of tests:	48 tests – Product No. 101.401-48 12 tests – Product No. 101.401-12
Number of tubes per test:	23 + 1
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C

This Product Description is only valid for Lot No. X92.

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. V87**).

One or both primers of the tubes detailed below have been exchanged, modified or added compared to the previous lot.

Tube	5'-primer	3'-primer	rationale
4	Exchanged	Exchanged	Primer pair exchanged to separate the A*11xx,24xx and the A*24xx2451 genotypes.
6	Added	Added	Added primer pairs for the A*9222 and A*8001 alleles.
11	Exchanged	Exchanged	Exchanged primer pair in exon 3 so that no A*03 alleles will be amplified.
20	Added/ modified	Added/ modified	Primers modified to separate the A*11xx,68xx and the A*1110,1116 genotypes. Primer pair added to separate the A*023502,0324 and the A*03xx,68xx genotypes.
21	Added	Added	Primer pair added for the A*6829 allele.
22	Added	Added	Primer pair added to separate the A*023502,0324 and the A*03xx,68xx genotypes.

Well **24** contains Negative Control primer pairs, that will amplify more than 90% 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.

Length of PCR product	105	200	105	80	75	80
5'-primer¹	164	340	440	45	45	43
	5'-CAC ^{3'}	5'-Agg ^{3'}	5'-TTA ^{3'}	5'-Tg g ^{3'}	5'-Tg g ^{3'}	5'-Tg g ^{3'}
3'-primer²	231	2nd I	507	59	58	57
	5'-TgC ^{3'}	5'-AAA ^{3'}	5'-TTg ^{3'}	5'-CTC ^{3'}	5'-ggC ^{3'}	5'-CTC ^{3'}
A*	+	+	+			
B*	+	+	+			
Cw*	+	+	+			
DRB1				+	+	
DRB3				+	+	
DRB5				+		
DQB1					+	
DPB1						+

¹The 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. Nucleotide numbering as in *Tissue Antigens* 1998, 51:II, 417-466. The sequence of the 3 terminal nucleotides of the primer is given.

²The 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 in the anti-sense direction. Nucleotide numbering as in *Tissue Antigens* 1998, 51:II, 417-466. The sequence of the 3 terminal nucleotides of the primer is given.

PRODUCT DESCRIPTION

HLA-A low resolution SSP typing

CONTENT

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

The primer solutions are pre-aliquoted into 0.2 ml PCR tubes. Each tube in the set contains a dried primer solution consisting of a specific primer mix, i.e. allele- and group-specific primers as well as a **control primer pair** matching non-allelic sequences.

PCR Master Mix complete with Taq, Taq polymerase, nucleotides, buffer, glycerol and cresol red, as well as PCR lids are included in the licensed kit.

PCR Master Mix without Taq, nucleotides, buffer, glycerol and cresol red, as well as PCR lids are included in the unlicensed kit.

23 + 1 PCR reactions with a reaction volume of 10 µl are performed per sample.

Note: The pellets in the tubes may vary in form and colour. This does not affect the performance of the product.

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

Wells 1 to 23 – HLA-A low resolution primers.

Well 24 – Negative Control.

The 24 well cut PCR plate is marked with 'A low X92'.

Well No. 1 is marked with '1'.

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 HLA-A low resolution typing kit. Thus, the interpretation of HLA-A low resolution typings is not influenced by other HLA class I alleles.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A alleles¹, i.e. **A*0101 to A*8001**, recognized by the HLA Nomenclature Committee in July 2007² will be amplified by the primers in the HLA-A low resolution SSP kit. The HLA-A alleles will be grouped into their corresponding serological specificities³.

¹The nucleotide sequence of the A*0128 allele is not yet retrievable from the GenBank or EMBL data banks.

²**Nomenclature for factors of the HLA system, 1998.** *Tissue Antigens* 1999; **53**: 407-446.
HLA-A alleles listed on the IMGT/HLA web page 2007-July-08, release 2.18.0, www.ebi.ac.uk/imgt/hla.

³The 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*2424 and A*2465 alleles will give rise to identical amplification patterns. These four alleles can be separated by the respective high resolution SSP primer sets.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The ambiguous HLA-A homo- and heterozygous combinations of allele groups based on the two first digits of the allele designation are available in the Score™ interpretation software. (In most cases these ambiguities can be resolved by the Olerup SSP™ HLA-A high resolution SSP sets.)

LICENSES

101.401-48/12 – licensed for PCR.

Notice to purchaser: Limited License.

The purchase price of this product includes limited, non-transferable rights under U.S. Patents 4,683,202, 4,683,195 and 4,965,188 and their foreign counterparts, owned by Roche Molecular Systems, Inc. and F. Hoffman-La Roche Ltd (“Roche”), to use only this amount of the product to practice the Polymerase Chain Reaction (“PCR”) Process described in said patents solely for the HLA Typing applications of the purchaser solely for organ or tissue or bone marrow transplantation, and explicitly excludes analysis of forensic evidence or parentage determination. The rights to use this product to perform and to offer commercial service for HLA Typing for organ or tissue transplantation using PCR, including reporting the results of the purchaser’s activities for a fee or other commercial consideration, is also hereby granted. Further information on purchasing licenses to practice PCR may be obtained by contacting in the United States, the Director of Licensing at Roche Molecular Systems, inc., 1145 Atlantic Avenue, Alameda, California 94501, and outside the United States, the PCR Licensing Manager, F. Hoffmann-La Roche Ltd, Grenzacherstr. 124, CH-4070 Basel, Switzerland.

101.401-48u/12u – not licensed for PCR.

Notice to purchaser: Disclaimer of License.

This product is optimized for use in the Polymerase Chain Reaction (“PCR”) Process which is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd (“Roche”). No license under these patents to use the PCR Process is conveyed expressly or by implication to the purchaser of this product. Further information on purchasing licenses to practice PCR may be obtained by contacting in the United States, the Director of Licensing at Roche Molecular Systems, inc., 1145 Atlantic Avenue, Alameda, California 94501.

101.401-48/12 and 101.401-48u/12u

These products use ARMS™ technology and is sold under license from Zeneca Limited. ARMS is the subject of European Patent No. 0332435, US Patent No. 5595890 and corresponding world-wide patents. ARMS is a trademark of Zeneca Limited.

GUARANTEE

Olerup SSP AB guarantees that the primers in the HLA-A low resolution typing kit have the specificities given in the Specificity and Interpretation Tables of the product insert and in the GenoVision version of the HELMBERG-SCORE™ software.

When stored at –20°C, the dried primers are stable for 22 months from the date of manufacture.

When stored at –20°C, the PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* are stable for 24 months from the date of manufacture.

The kit is shipped at ambient temperature.

PROTOCOL

DNA EXTRACTION

Extracted, highly pure DNA is needed for SSP typings. We recommend isolation of DNA using GenoPrep B200 or GenoPrep B350 cartridges on the GenoM™-6 robotic workstation (GenoVision Europe Tel: +43 1 710 15 00 or GenoVision Inc. USA Tel: +1 610 430 88 41; <http://www.genovision.com>). Using GenoM™-6-extracted DNA ACD, EDTA and heparinised blood can be used as starting material. Because of its high purity, GenoM™-6-extracted DNA can be diluted when used in combination with *Olerup* SSP™ products. The recommended DNA concentration is 15 ng/μl.

Alternatively – BUT DO NOT USE HEPARINISED BLOOD WITH THESE METHODS - the DNA can be extracted using trimethylammoniumbromide salts (DTAB/CTAB) or by salting out. Dissolve the extracted DNA in dH₂O.

IMPORTANT:

Optimal DNA concentration using: GenoM™-6-extracted DNA, 15 ng/μl.

DNA extracted by other methods, 30 ng/μl.

Concentration exceeding 50 ng/μl will increase the risk for nonspecific amplifications and weak extra bands, especially for HLA Class I high resolution SSP typings.

PCR AMPLIFICATION

101.401-48/12 – licensed for PCR

For one HLA-A low resolution typing, begin by adding to well No. 24, i.e. the well with the negative control primer pairs:

7 μl dH₂O

3 μl PCR Master Mix complete with *Taq*,

then add at room temperature in a 0.5 ml tube:

27 x 2 μl = 54 μl DNA (30 ng/μl)

27 x 3 μl = 81 μl PCR Master Mix complete with *Taq* – mix well

before taking your aliquot

27 x 5 μl = 135 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-H₂O mixture into each of the 23 wells of an HLA-A low resolution typing, i.e. wells 1 to 23. **Well No. 1 of the 24 well PCR plate is marked with '1'**. Close the 24 well PCR plate with the provided lids.

101.401-48u/12u – not licensed for PCR

For one HLA-A low resolution typing, begin by adding at room temperature in a 0.5 ml tube:

28 x 3 μl = 84 μl PCR Master Mix without *Taq* – mix well before taking your aliquot

2.2 μl *Taq* polymerase (5 units/μl)

Mix well, dispense 3 μ l of the PCR Master Mix-*Taq* mixture from the 0.5 ml tube into well No. 24, i.e. the well with the negative control primer pairs. Then add 7 μ l dH₂O to well 24.

Then add at room temperature to the 0.5 ml tube containing $84 + 2.2 - 3 = 83,2$ μ l PCR Master Mix-*Taq* mixture:

$$27 \times 2 \mu\text{l} = 54 \mu\text{l DNA (30 ng}/\mu\text{l)}$$

$$27 \times 5 \mu\text{l} - 2,2 \mu\text{l} = 132.8 \mu\text{l dH}_2\text{O}$$

Mix well, dispense 10 μ l of the DNA-PCR Master Mix-*Taq*-H₂O mixture into each of the 23 wells of an HLA-A low resolution typing, i.e. wells 1 to 23. **Well No. 1 of the 24 well PCR plate is marked with '1'**. Close the 24 well PCR plate with the provided lids.

Use a 96 well thermal cycler with a heated lid. The temperature gradient across the heating block should be < 1°C.

PCR cycling parameters:

1. 1 cycle	94°C	2 min	denaturation
2. 10 cycles	94°C	10 sec.	denaturation
	65°C	60 sec.	annealing and extension
3. 20 cycles	94°C	10 sec.	denaturation
	61°C	50 sec.	annealing
	72°C	30 sec.	extension

The same PCR cycling parameters are used for all the Olerup SSP kits.

AGAROSE GEL ELECTROPHORESIS

Prepare a 2% (w/v) agarose gel in 0.5 x TBE buffer. Dissolve the agarose by boiling in a microwave oven. Let the gel solution cool to 60°C. Stain the gel prior to casting with ethidium bromide (10 mg/ml), 5 μ l per 100 ml gel solution. For maximal ease of handling use our ethidium bromide dropper bottles (Product No. 103.301-10), 1 drop of ethidium bromide solution per 50-75 ml of gel. **Note: Ethidium bromide is a powerful carcinogen.**

Load the PCR products, preferably using an 8-channel pipette. Load a DNA size marker (100 base pair ladder, Product No. 103.201-100) in one well per row.

Run the gel in 0.5 x TBE buffer, without re-circulation of the buffer, for 15-20 minutes at 8-10 V/cm.

DOCUMENTATION AND INTERPRETATION

Put the gel on a UV transilluminator and document by photography.

Record the presence and absence of specific PCR products. The relative lengths of the specific PCR products are helpful in the interpretation of the results.

Record the presence and relative lengths of the internal positive control bands. The differently sized control bands will help in the correct orientation of the typing as well as in kit identification.

Lanes without either control band or specific PCR products should be repeated.

Interpret the typings with the ***lot-specific Interpretation and Specificity Tables***.

INTERPRETATION SOFTWARE

The interpretation software (Product No. 110.101) can be helpful in the interpretation of the typings.

PCR MASTER MIXES

The PCR Master Mix complete with *Taq* contains:

<i>Taq</i> polymerase	0.4 unit per 10 μ l SSP reaction
nucleotides	final concentration of each dNTP is 200 μ M
PCR buffer	final concentrations: 50 mM KCl, 1.5 mM MgCl ₂ , 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin
glycerol	final concentration of glycerol is 5%
cresol red	final concentration of cresol red is 100 μ g/ml

The same PCR Master Mix complete with *Taq* is used for all the licensed *Olerup* SSP kits.

The PCR Master Mix without *Taq* contains:

nucleotides	final concentration of each dNTP is 200 μ M
PCR buffer	final concentrations: 50 mM KCl, 1.5 mM MgCl ₂ , 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin
glycerol	final concentration of glycerol is 5%
cresol red	final concentration of cresol red is 100 μ g/ml

The same PCR Master Mix without *Taq* is used for all the unlicensed *Olerup* SSP kits.

The PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* can be shipped at ambient temperature.

When stored at -20°C , the PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* are stable for 24 months from the date of manufacture.

Vials with the PCR Master Mixes can be kept at $+4^{\circ}\text{C}$ for 4 weeks, but the PCR Master Mixes are then no longer stable for 24 months.

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	Approx. size of spec. PCR product ¹	Size of control band ²	HLA-A serology ³	Amplified HLA-A alleles ^{4,10}
1 ⁵	120, 145 bp	800 bp	A1, A36	01010101-0104N, 0106-0127N, 3601-3604
2 ⁶	135, 200, 255 bp	1070 bp	A2	02010101-0222, 0224-023501, 023503-0247, 0249-0297, 0299, 9201-9221, 9223-9226
3 ⁷	205, 235 bp	1070 bp	A1, A3, A11, A32, A34, A36	0112, 0119, 0121, 03010101-0317, 0319-0330, 1125, 3204, 3402-3404, 3407, 3408, 3602
4 ⁷	190 bp	800 bp	A1, A2, A3, A11, A24, A26, A30, A36	01010101-0104N, 0106, 0107, 0109-0111N, 0113, 0116N-0118N, 0120-0127N, 0278, 0312, 0318, 110101-1132, 2619, 3008, 3604
5	160, 535 bp	800 bp	A3, A23, A24, A31, A32	0330, 2301-2316, 24020101-2411N, 2413-2415, 2417-2479, 3108, 3205, 3213
6	165, 200 bp	800 bp	A2, A23, A24, A29, A80	9222, 2301-2316, 2405, 2424, 2465, 2907, 8001
7	175, 205 bp	1070 bp	A2, A23, A24, A26	021701-021702 ^{weakly} , 2314, 24020101-2411N, 2413, 2417-2450, 2454-2456, 2458-2463, 2465-2479, 2616
8	165, 200 bp	800 bp	A2, A3, A11, A25, A26, A32, A34, A66, A68, A69	0255, 0324, 1110, 250101-2506, 260101-2606, 2608-2615, 2617, 2618, 2620-2635, 3215, 3401-3408, 6601-6606, 680101-6840, 6901
9 ⁵	75 bp	800 bp	A2, A25, A32	9224, 250101-2506, 320101-3202, 3204, 3206-

10⁵	85 bp	1070 bp	A26, A33	3215 260101-2602, 2604, 260701-2618, 2620, 2622- 2629, 2632-2635, 3313
11^{5,7,8}	80, 175, 500 bp	1070 bp	A1, A2, A11, A24, A26, A31, A34, A66	0113, 0227, 110101-1132, 2419, 2444, 2603, 2606, 2621, 3103, 3401-3408, 6601, 6604-6606
12	185 bp	800 bp	A11, A25, A26, A31, A34, A43, A66	1111, 2506, 2609, 3103, 3104, 3401-3408, 4301, 6602, 6603
13⁷	175, 225 bp	1070 bp	A1, A2, A3, A25, A26, A34, A43, A66	0113, 0234-023503, 0256, 0262, 9203, 03010101- 0307, 0309-0311N, 0313- 0330, 250101-2505, 260101-260105, 2602 ^{weakly} , 2603, 2605- 2608, 2610-2633, 2635, 3408, 4301, 6601, 6604- 6606
14	200, 240 bp	1070 bp	A29, A33	29010101-2916, 3313
15⁵	90 bp	1070 bp	A30	300101-3004, 3006-3022
16	180, 210 bp	1070 bp	A29, A31	2914, 310102-3118
17	180 bp	1070 bp	A29, A32	2913, 320101-3203, 3205- 3215
18	200 bp	1070 bp	A33, A68	3301, 330301-3313, 6829
19	160, 200 bp	800 bp	A74	7401-7412N
20	155, 240 bp	800 bp	A2, A210, A25, A30, A68	0210, 0234-023503, 0239, 0246, 0248, 0250, 0252, 0256, 0262, 0270, 0273, 0278, 0293, 0295, 9203, 9210, 9214, 9217, 9222, 2505, 3008, 680101-6840
21	240, 375 bp	800 bp	A2, A26, A68, A69	0255, 2622, 6829, 6901
22^{5,9}	85, 240 bp	800 bp	A2, A30, A36	0234-023503, 0246, 0248, 0256, 0262, 0270, 0278, 9203, 3008, 3601-3604
23⁵	75, 160 bp	800 bp	A2, A26, A68, A80	0255, 2603, 2605, 2606, 2621, 2630, 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 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.

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 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 low resolution typing.

In addition, tubes 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.

PLEASE NOTE: All the SSP kits, except the B*37, B*41, B*42, B*46, B*47, B*48, B*49, B*50, B*53, B*67, B*78, B*81 and B*82 kits and the Cw*01, Cw*02, Cw*08, Cw*12, Cw*14, Cw*15, Cw*16, Cw*17 and Cw*18 kits, from *Olerup* SSP AB can be uniquely identified by the number of tubes and the kit-specific pattern of the two differently sized control bands.

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 used the information published in *Tissue Antigens* 1999: **54**: 409-437, also available at the www.worldmarrow.org web site and 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.

The nucleotide sequence of the A*0128 allele is not yet retrievable from the GenBank or EMBL data banks.

⁵ Specific PCR fragments shorter than 125 base pairs have a lower intensity than longer PCR bands.

⁶ The primer pairs in vial 2 will in most samples give rise to two specific PCR fragments.

⁷ Primer mixes 3, 4, 11 and 13 may give rise to nonspecific amplifications.

⁶ The primer pairs in vial 11 will in most samples give rise to two to three specific PCR fragments.

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*2424 and A*2465 alleles will give rise to identical amplification patterns. These four alleles can be separated by the respective high resolution SSP primer sets.

'w', might be weakly amplified.

INTERPRETATION TABLE													
HLA-A low resolution SSP typing													
Amplification patterns of the A*0101 to A*8001 alleles													
		Tube⁶											
		1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.		120	135	205	190	160	165	175	165	75	85	80	185
PCR product		145	200	235		535	200	205	200			175	
			255									500	
Length of int.		800	1070	1070	800	800	800	1070	800	800	1070	1070	800
pos. control ¹													
5'-primer(s) ²		98	78	363	98	144	106	98	98	266	257	302	103
		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'}
		123	106		413	317	176	368	102	266	261	385	423
		5'-AgT ^{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'}
			144				368		413				
			5'-gCA ^{3'}				5'-gTT ^{3'}		5'-CCg ^{3'}				
			144										
			5'-TCA ^{3'}										
3'-primer(s) ³		203	240	527	256	265	256	259	259	302	299	341	257
		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'}
			292	527	559	570	292	502	538			521	559
			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'}
				555			502	539					559
				5'-CCA ^{3'}			5'-CTg ^{3'}	5'-TCT ^{3'}					5'-CgT ^{3'}
				555			538						
				5'-gCA ^{3'}			5'-CAg ^{3'}						
Tube No.		1	2	3	4	5	6	7	8	9	10	11	12
HLA-A allele ⁴	ser ⁵												
*01010101-0104N, 0106, 0107, 0109-0111N, 0116N-0118N, 0120, 0122N-0127N	A1, null	+			+								
*0108, 0114, 0115N	1	+											
*0112, 0119	-	+		+									
*0113	-	+			+							+	
Tube No.		1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE											
HLA-A low resolution SSP typing											
Amplification patterns of the A*0101 to A*8001 alleles											
Tube⁶											
13	14	15	16	17	18	19	20	21	22	23	
175	200	90	180	180	200	160	155	240	85	75	Length of spec.
225	240		210			200	240	375	240	160	PCR product
1070	1070	1070	1070	1070	1070	800	800	800	800	800	Length of int.
											pos. control ¹
98	98	363	97	180	98	180	78	28	78	176	5'-primer(s) ²
5'-CTT ^{3'}	5'-CAC ^{3'}	5'-ATA ^{3'}	5'-TCA ^{3'}	5'-TTT ^{3'}	5'-CAC ^{3'}	5'-TTT ^{3'}	5'-TCT ^{3'}	5'-TCg ^{3'}	5'-TCT ^{3'}	5'-gCA ^{3'}	
423							391	261	527	261	
5'-gCT ^{3'}							5'-ACg ^{3'}	5'-AAC ^{3'}	5'-TgC ^{3'}	5'-AAC ^{3'}	
							391				
							5'-ACg ^{3'}				
282	257	411	238	317	256	299	265	97	265	292	3'-primer(s) ³
5'-gAC ^{3'}	5'-gCA ^{3'}	5'-TCA ^{3'}	5'-CCT ^{3'}	5'-ggA ^{3'}	5'-CCC ^{3'}	5'-CCA ^{3'}	5'-CCC ^{3'}	5'-ggT ^{3'}	5'-CCC ^{3'}	5'-gTT ^{3'}	
559	299		265		259	299	282	355	282	292	
5'-CCg ^{3'}	5'-TCg ^{3'}		5'-CCC ^{3'}		5'-gTT ^{3'}	5'-CCA ^{3'}	5'-gAC ^{3'}	5'-gAC ^{3'}	5'-gAC ^{3'}	5'-gTg ^{3'}	
						341	506		570	299	
						5'-CgT ^{3'}	5'-TgT ^{3'}		5'-CAC ^{3'}	5'-TCT ^{3'}	
13	14	15	16	17	18	19	20	21	22	23	Tube No.
											ser ⁵
											HLA-A allele ⁴
											A1, null
											*01010101-0104N, 0106, 0107, 0109-0111N, 0116N 0118N, 0120, 0122N- 0127N
											1
											*0108, 0114, 0115N
											-
											*0112, 0119
											-
											*0113
+											-
13	14	15	16	17	18	19	20	21	22	23	Tube No.

Length of spec.		120	135	205	190	160	165	175	165	75	85	80	185
PCR product		145	200	235		535	200	205	200			175	
			255									500	
Tube No.		1	2	3	4	5	6	7	8	9	10	11	12
*0121	-	+		+	+								
*02010101-0209, 0211-0216, 0218-0222, 0224-0226, 0228-0233, 0236-0238, 0240-0245, 0247, 0249, 0251, 0253N, 0254, 0257-0261, 0263-0269, 0271, 0272, 027401-0277, 0279-0292, 0294N, 0296, 0297, 0299, 9201, 9202, 9204-9209, 9211-9213N, 9215, 9216, 9218-9221, 9223, 9225N, 9226	A2, A203, null		+										
*0210, 0239, 0250, 0252, 0273, 0293, 0295, 9210, 9214, 9217	A210		+										
*021701-021702	A2		+					W					
*0227	-		+									+	
*0234, 023501, 023503, 0256, 0262, 9203	-		+										
*023502	-												
*0246, 0270	-		+										
*0248	-												
*0255	-		+						+				
*0278	-		+		+								
*9222	-						+						
*9224	-		+							+			
*03010101-0307, 0309-0311N, 0313-0317, 0319-0323, 0325-0329	A3			+									
*0308	-			+									
*0312	-			+	+								
*0318, 2619 ⁷	-				+								
*0324	-			+					+				
*0330	-			+		+							
*110101-1109, 1112-1124, 1126-1132	A11				+							+	
*1110	-				+				+			+	
*1111	-				+							+	+
Tube No.		1	2	3	4	5	6	7	8	9	10	11	12

175	200	90	180	180	200	160	155	240	85	75		Length of spec.
225	240		210			200	240	375	240	160		PCR product
13	14	15	16	17	18	19	20	21	22	23		Tube No.
											-	*0121
											A2, A203, null	*02010101-0209, 0211-0216, 0218-0222, 0224-0226, 0228-0233, 0236-0238, 0240-0245, 0247, 0249, 0251, 0253N, 0254, 0257-0261, 0263-0269, 0271, 0272, 027401-0277, 0279-0292, 0294N, 0296, 0297, 0299, 9201, 9202, 9204-9209, 9211-9213N, 9215, 9216, 9218-9221, 9223, 9225N, 9226
							+				A210	*0210, 0239, 0250, 0252, 0273, 0293, 0295, 9210, 9214, 9217
											A2	*021701-021702
											-	*0227
+							+		+		-	*0234, 023501, 023503, 0256, 0262, 9203
+							+		+		-	*023502
							+		+		-	*0246, 0270
							+		+		-	*0248
								+		+	-	*0255
							+		+		-	*0278
							+				-	*9222
											-	*9224
+											A3	*03010101-0307, 0309-0311N, 0313-0317, 0319-0323, 0325-0329
											-	*0308
											-	*0312
+											-	*0318, 2619 ⁷
+											-	*0324
+											-	*0330
											A11	*110101-1109, 1112-1124, 1126-1132
											-	*1110
											-	*1111
13	14	15	16	17	18	19	20	21	22	23		Tube No.

Length of spec.		120	135	205	190	160	165	175	165	75	85	80	185
PCR product		145	200	235		535	200	205	200			175	
			255									500	
Tube No.		1	2	3	4	5	6	7	8	9	10	11	12
*1125	-			+	+							+	
*2301-2313, 2315, 2316	A23					+	+						
*2314, 2405, 2424, 2465 ⁸	-					+	+	+					
*24020101-2404, 2406-2411N, 2413, 2417, 2418, 2420-2423, 2425-2443, 2445N-2450, 2454-2456, 2458-2463, 2466-2479	A24					+		+					
*2414, 2415, 2451-2453, 2457, 2464	A24					+							
*2419, 2444	-					+		+				+	
*250101-2504	A25								+	+			
*2505	-								+	+			
*2506	-								+	+			+
*260101-260105, 2608, 2610-2615, 2617, 2618, 2620, 2623-2629, 2632, 2633, 2635	A26, null								+		+		
*2602	A26								+		+		
*2603, 2606, 2621	A26								+			+	
*2604	A26								+		+		
*2605, 2630	A26								+				
*260701-260702	A26										+		
*2609	A26								+		+		+
*2616	-							+			+		
*2622	-								+		+		
*2631	-								+				
*2634	-								+		+		
*29010101-2906, 2908N-2912, 2915, 2916	A29												
*2907	-						+						
*2913	-												
*2914	-												
*300101-3004, 3006, 3007, 3009-3022	A30												
*3008	-				+								
*310102-3102, 3105-3107, 3109-3118	A31												
Tube No.		1	2	3	4	5	6	7	8	9	10	11	12

175	200	90	180	180	200	160	155	240	85	75		Length of spec.
225	240		210			200	240	375	240	160		PCR product
13	14	15	16	17	18	19	20	21	22	23		Tube No.
											-	*1125
											A23	*2301-2313, 2315, 2316
											-	*2314, 2405, 2424, 2465 ⁸
											A24	*24020101-2404, 2406-2411N, 2413, 2417, 2418, 2420-2423, 2425-2443, 2445N-2450, 2454-2456, 2458-2463, 2466-2479
											A24	*2414, 2415, 2451-2453, 2457, 2464
											-	*2419, 2444
+											A25	*250101-2504
+							+				-	*2505
											-	*2506
+											A26, null	*260101-260105, 2608, 2610-2615, 2617, 2618, 2620, 2623-2629, 2632, 2633, 2635
W											A26	*2602
+										+	A26	*2603, 2606, 2621
											A26	*2604
+										+	A26	*2605, 2630
+											A26	*260701-260702
											A26	*2609
+											-	*2616
+								+			-	*2622
+											-	*2631
											-	*2634
	+										A29	*29010101-2906, 2908N-2912, 2915, 2916
	+										-	*2907
	+			+							-	*2913
	+		+								-	*2914
		+									A30	*300101-3004, 3006, 3007, 3009-3022
		+					+		+		-	*3008
			+								A31	*310102-3102, 3105-3107, 3109-3118
13	14	15	16	17	18	19	20	21	22	23		Tube No.

Length of spec.		120	135	205	190	160	165	175	165	75	85	80	185
PCR product		145	200	235		535	200	205	200			175	
			255									500	
Tube No.		1	2	3	4	5	6	7	8	9	10	11	12
*3103	-											+	+
*3104	A31												+
*3108	-					+							
*320101-3202, 3206-3212, 3214	A32									+			
*3203	-												
*3204	A3			+						+			
*3205	-					+							
*3213	-					+				+			
*3215	-								+	+			
*3301, 330301-3312	A33												
*3313	-										+		
*3401, 3405, 3406	A34								+			+	+
*3402-3404, 3407	-			+					+			+	+
*3408	-			+					+			+	+
*3601, 3603	A36	+											
*3602	-	+		+									
*3604	-	+			+								
*4301	A43												+
*6601, 6604-6606	A66								+			+	
*6602, 6603	A66								+				+
*680101-6804, 6806-6814, 6816-6819, 6821-6828, 6830-6840	A68								+				
*6805, 6815, 6820	-								+				
*6829	-								+				
*6901	A69								+				
*7401-7412N	A74												
*8001	A80						+						
HLA-A allele ⁴	ser ⁵												
Tube 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 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 low resolution SSP typing. In addition, tubes 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.

175	200	90	180	180	200	160	155	240	85	75		Length of spec.
225	240		210			200	240	375	240	160		PCR product
13	14	15	16	17	18	19	20	21	22	23		Tube No.
			+								-	*3103
			+								A31	*3104
			+								-	*3108
				+							A32	*320101-3202, 3206-3212, 3214
				+							-	*3203
											A3	*3204
				+							-	*3205
				+							-	*3213
				+							-	*3215
					+						A33	*3301, 330301-3312
	+				+						-	*3313
											A34	*3401, 3405, 3406
											-	*3402-3404, 3407
+											-	*3408
									+		A36	*3601, 3603
									+		-	*3602
									+		-	*3604
+											A43	*4301
+											A66	*6601, 6604-6606
											A66	*6602, 6603
							+				A68	*680101-6804, 6806-6814, 6816-6819, 6821-6828, 6830-6840
							+			+	-	*6805, 6815, 6820
					+		+	+			-	*6829
								+			A69	*6901
						+					A74	*7401-7412N
										+	A80	*8001
											ser ⁵	HLA-A allele ⁴
13	14	15	16	17	18	19	20	21	22	23		Tube No.

²The nucleotide position, in the 1st, 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as in *Tissue Antigens* 1998, 51:II, 417-466. 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 in *Tissue Antigens* 1998, 51:II, 417-466. The sequence of the 3 terminal nucleotides of the primer is given.

⁴The nucleotide sequence of the A*0128 allele is not yet retrievable from the GenBank or EMBL data banks.

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

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 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 sequence of the A*3005 allele has been shown to be identical to A*3004.

The sequence of the A*31011 allele has been shown to be identical to A*31012.

The sequence of the A*3302 allele has been shown to be identical to A*3303.

⁵The serological reactivity of all HLA-A alleles is not known. In this table we used the information published in *Tissue Antigens* 1999: **54**: 409-437, also available at the www.worldmarrow.org web site and also inferred the serological grouping from the naming of the sequence-defined allele.

⁶The primer pairs in vial 2 will in most samples give rise to two specific PCR fragments.

The primer pairs in vial 11 will in most samples give rise to two to three specific PCR fragments.

⁷The 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.

⁸The A*2314, A*2405, A*2424 and A*2465 alleles will give rise to identical amplification patterns. These four alleles can be separated by the respective high resolution SSP primer sets. 'w', might be weakly amplified.

CELL LINE VALIDATION SHEET																			
HLA-A low resolution primer set																			
				Lot No.:	Tube														
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	cell line	A*	A*		200626301	200626302	200626303	200731404	200626305	200735006	200626307	200626308	200626309	200626310	200735011	200626312	200626313	200626314	200626315
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			-	+	-	-	-	-	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											
				Tube							
				16	17	18	19	20	21	22	23
				Lot No.:							
				200626316	200626317	200626318	200626319	200731420	200733421	200731422	200733423
	cell line	A*	A*								
1	9001 SA	*2402		-	-	-	-	-	-	-	-
2	9280 LK707	*0201		-	-	-	-	-	-	-	-
3	9011 E4181324	*0101		-	-	-	-	-	-	-	-
4	9275 GU373	*3001		-	-	-	-	-	-	-	-
5	9009 KAS011	*0101		-	-	-	-	-	-	-	-
6	9353 SM	*0201	*2603	-	-	-	-	-	-	-	+
7	9020 QBL	*2601		-	-	-	-	-	-	-	-
8	9007 DEM	*0201		-	-	-	-	-	-	-	-
9	9026 YAR	*2601		-	-	-	-	-	-	-	-
10	9107 LKT3	*2402		-	-	-	-	-	-	-	-
11	9051 PITOUT	*2902		-	-	-	-	-	-	-	-
12	9052 DBB	*0201		-	-	-	-	-	-	-	-
13	9067 BTB	*0201		-	-	-	-	-	-	-	-
14	9071 OLGA	*3101		+	-	-	-	-	-	-	-
15	9075 DKB	*2402		-	-	-	-	-	-	-	-
16	9037 SWEIG007	*2902		-	-	-	-	-	-	-	-
17	9008 WILJON	*2501		-	-	-	-	-	-	-	-
18	9257 32367	*3303	*7401	-	-	+	+	-	-	-	-
19	9038 BM16	*0201		-	-	-	-	-	-	-	-
20	9059 SLE005	*0201		-	-	-	-	-	-	-	-
21	9064 AMALA	*0217		-	-	-	-	-	-	-	-
22	9056 KOSE	*0201		-	-	-	-	-	-	-	-
23	9124 IHL	*0201	*3401	-	-	-	-	-	-	-	-
24	9035 JBUSH	*3201		-	+	-	-	-	-	-	-
25	9049 IBW9	*3301		-	-	+	-	-	-	-	-
26	9285 WT49	*0205		-	-	-	-	-	-	-	-
27	9191 CH1007	*2410	*2901	-	-	-	-	-	-	-	-
28	9320 BEL5GB	*0201	*2902	-	-	-	-	-	-	-	-
29	9050 MOU	*2902		-	-	-	-	-	-	-	-
30	9021 RSH	*3001	*6802	-	-	-	-	+	-	-	-
31	9019 DUCAF	*3002		-	-	-	-	-	-	-	-
32	9297 HAG	*0201		-	-	-	-	-	-	-	-
33	9098 MT14B	*3101		+	-	-	-	-	-	-	-
34	9104 DHIF	*3101		+	-	-	-	-	-	-	-
35	9302 SSTO	*3201		-	+	-	-	-	-	-	-
36	9024 KT17	*0206	*1101	-	-	-	-	-	-	-	-
37	9065 HHKB	*0301		-	-	-	-	-	-	-	-
38	9099 LZL	*0217		-	-	-	-	-	-	-	-
39	9315 CML	*0101	*0301	-	-	-	-	-	-	-	-
40	9134 WHONP199	*0207	*3001	-	-	-	-	-	-	-	-
41	9055 H0301	*0301		-	-	-	-	-	-	-	-
42	9066 TAB089	*0207		-	-	-	-	-	-	-	-
43	9076 T7526	*0207		-	-	-	-	-	-	-	-
44	9057 TEM	*6601		-	-	-	-	-	-	-	-
45	9239 SHJO	*2301	*2402	-	-	-	-	-	-	-	-
46	9013 SCHU	*0301		-	-	-	-	-	-	-	-
47	9045 TUBO	*0216	*0301	-	-	-	-	-	-	-	-
48	9303 TER-ND	*0201	*1101	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP™ HLA-A low resolution SSP

Product number: 101.401-48/12 – licensed for PCR
101.401-48u/12u – not licensed for PCR

Lot number: X92

Expiry date: 2009-June-01

Number of tests: 48 tests – Product No. 101.401-48
12 tests – Product No. 101.401-12

Number of tubes per test: 23 + 1

Tube specifications:

Tube No.	Production No.	Tube No.	Production No.	Tube No.	Production No.
1	2006-263-01	9	2006-263-09	17	2006-263-17
2	2006-263-02	10	2006-263-10	18	2006-263-18
3	2006-263-03	11	2007-350-11	19	2006-263-19
4	2007-314-04	12	2006-263-12	20	2007-314-20
5	2006-263-05	13	2006-263-13	21	2007-334-21
6	2007-350-06	14	2006-263-14	22	2007-314-22
7	2006-263-07	15	2006-263-15	23	2007-334-23
8	2006-263-08	16	2006-263-16		

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

Additional 5'-primers in primer solutions 8 and 10 were tested by separately adding one 3'-primer. Additional 3'-primers in primer solutions 3, 4, 8, 14, 16, 18, 19 and 23 were tested by separately adding one 5'-primer. Additional 5'- and 3'-primers in primer solutions 5, 7, 12 and 22 were tested by separately adding one 3'-primer, respectively one 5'-primer.

The negative control primer pairs, **Production No. 2006-148-01**, can detect contamination with PCR products diluted 10^{-7} .

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

Date of approval: 2007-September-09

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: Olerup SSP™ HLA-A low resolution
Product number: 101.401-48/12, 101.401-48u/12u
Lot number: X92

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 9001:2000 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
2007-September-09

Olle Olerup
Managing Director

HLA-A low resolution
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WARRANTY

Olerup SSP AB warrants its products to the original purchaser against defects in materials and workmanship under normal use and application. *Olerup* SSP AB's sole obligation under this warranty shall be to replace, at no charge, any product that does not meet the performance standards stated on the product specification sheet.

This warranty applies only to products that have been handled and stored in accordance with *Olerup* SSP AB's recommendations, and does not apply to products that have been the subject of alternation, misuse, or abuse.

All claims under this warranty must be directed to *Olerup* SSP AB in writing and must be accompanied by a copy of the purchaser's invoice. This warranty is in lieu of all other warranties, expressed or implied, including the warranties of merchantability and fitness for a particular purpose. In no case shall *Olerup* SSP AB be liable for incidental or consequential damages.

This product may not be reformulated, repacked or resold in any form without the written consent of *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

Handle all samples as if capable of transmitting disease. All work should be performed wearing gloves and appropriate protection.

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PCR™ is a trademark of F. Hoffmann-La Roche Ltd.
ARMS™ is a trademark of Zeneca Ltd.

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