

Olerup SSP™ HLA-A*01

Product number: 101.411-24/06– licensed for PCR
101.411-24u/06u – not licensed for PCR
Lot number: X98
Expiry date: 2009-June-01
Number of tests: 24 test – Product No. 101.411-24
6 tests – Product No. 101.411-06
Number of tubes per test: 24
Storage - pre-aliquoted primers: dark at -20°C
- PCR Master Mix: -20°C

This Product Description is only valid for Lot No. X98.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP™ HLA-A*01 LOT

The HLA-A*01 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous Olerup SSP™ HLA-A*01 lot was made (Lot No. V64).

Three tubes have been added to the HLA-A*01 kit,
wells **22 to 24**.

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

Tube	5'-primer	3'-primer	rationale
5	Modified	Modified	Increased specificity of specific primer pair.
8	Exchanged	Exchanged	Primer pair exchanged for the A*0123 allele.
9	-	Added	Primer added for the A*0127N allele.
13	-	Exchanged	Increased resolution.
20	Added	Added	Primer pair added for the A*0126 allele.
22	New	New	New primer pair for A*0122N allele.
23	New	New	New primer pair for A*0124 allele.
24	New	New	New primer pair for A*0125 allele.

Changes in revision R01 compared to R00:

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

Changes in revision R02 compared to R01:

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

PRODUCT DESCRIPTION

HLA-A*01 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*0101 to A*0127N alleles.

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.

24 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.

STRIP LAYOUT

Each test consists of 24 PCR reactions in a 24 well cut PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24

The 24 well cut PCR plate is marked with 'A*01 X98'.

Well No. 1 is marked with '1'.

The PCR plates are heat-sealed with a PCR-compatible foil.

Please note: When removing each 24 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

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

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*01 alleles¹, i.e. **A*0101 to A*0127N**, recognized by the HLA Nomenclature Committee in July 2007² will give rise to unique amplification patterns by the primers in the HLA-A*01 subtyping kit³.

The HLA-A*01 subtyping kit cannot distinguish the A*01010101, A*010102 to A*010104 alleles.

¹The nucleotide sequence of the A*0128 allele is not yet retrievable.

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-09, release 2.18.0, www.ebi.ac.uk/imgt/hla.

³The amplification patterns of the A*01010102N and A*0115N alleles only differ by the different lengths of the specific PCR products generated by primer mix 16.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 27 HLA-A*01 alleles can be combined in 378 homozygous and heterozygous combinations. 203 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products generated by primer mixes 16 was considered in these calculations.

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

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++----- -----+ -+-+----- 01010102,0126 = 0115N,0126
++----- -----+ -+----+--- 01010102,0122N = 0115N,0122N
++----- -----+ -+-----+- 01010102,0124 = 0115N,0124
++----- -----+ -+-----+ 01010102,0125 = 0115N,0125
++----- -----+ -+----- 0101,01010102 = 0101,0115N =
01010102,01010102 = 01010102,0115N
++----- ----- ++----- 0101,0116N = 0116N,0116N
++----- ----- -+-+----- 0101,0118N = 0118N,0118N
++----- ----- -+-+----- 0101,0126 = 0126,0126
++----- ----- -+----+--- 0101,0122N = 0122N,0122N
++----- ----- -+-----+- 0101,0124 = 0124,0124
++----- ----- -+-----+ 0101,0125 = 0125,0125
-+----- -----+-+ -+-+-----+ 0112,0119 = 0114,0119 = 0119,0119
-+----- -----+-+ -+-----+ 0112,0112 = 0112,0114

(0101 = 01010101 + 010102 to 010104)

LICENSES

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Notice to purchaser: Limited License.

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101.411-24/06 and 101.411-24u/06u

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*01 subtyping kit have 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

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For one HLA-A*01 subtyping, add at room temperature in a 0.5 ml tube:

28 x 2 μl = 56 μl DNA (30 ng/μl)

28 x 3 μl = 84 μl PCR Master Mix complete with *Taq* – mix well before taking your aliquot

28 x 5 μl = 140 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-H₂O mixture into each of the 24 wells of an HLA-A*01 subtyping. **Well No. 1 of the 24 well PCR plate is marked with '1'**. Close the 24 well PCR strip with the provided lids.

101.411-24u/06u – not licensed for PCR

For one HLA-A*01 subtyping, add at room temperature in a 0.5 ml tube:

28 x 2 μl = 56 μl DNA (30 ng/μl)

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)

28 x 5 μl – 2.2 μl = 137.8 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-*Taq*-H₂O mixture into each of the 24 wells of an HLA-A*01 subtyping. **Well No. 1 of the 24 well PCR plate is marked with '1'**. Close the 24 well PCR strip 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 µl 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*01 SSP subtyping

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

Primer Mix	Approx. size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*01 alleles ³	Other amplified HLA-A alleles ⁴
1	235 bp	800 bp	01010101-0102, 0104N, 0106, 0107, 0109-0111N, 0113, 0115N, 0116N-0118N, 0121-0127N	0318, 110101-1103, 1105-1125, 1128-1132, 3604
2⁵	145 bp	1070 bp	01010101-010104, 0103, 0104N, 0106-0119, 0121-0127N	3601-3604
3⁵	120 bp	800 bp	0102, 0120	
4	305 bp	1070 bp	0103	1126, 2632, 3313, 3603, 7410
5⁶	470 bp	1070 bp	0104N	0321N, 1121N, 2307N, 2411N
6	210 bp	1070 bp	0109	
7⁷	215 bp	800 bp	0106	0305, 1124, 1125, 1131, 300101-3003, 3007-3016, 3018-3022, 3103, 3104, 3402-3404, 3407, 3408, 8001
8⁵	110 bp	1070 bp	0107, 0123	
9	235 bp	1070 bp	0108, 0127N	
10	155 bp	800 bp	0110	1114 ^{weakly} , 8001 ^{weakly}
11⁵	275 bp	800 bp	0111N	
12^{5,8}	85 bp	800 bp		3601-3604
13	205 bp	1070 bp	0112, 0119, 0121	0302, 0307 ^{weakly} , 0310, 1131 ^{weakly} , 2451, 3004 ^{weakly} , 3006 ^{weakly} , 3009 ^{weakly} , 3017 ^{weakly} ,

				3103 ^{weakly} , 3104 ^{weakly}
14 ⁵	120 bp	1070 bp	0113, 0117	
15 ⁶	235 bp	1070 bp	0112, 0114, 0119	03010101-0317, 0319-0330, 1104, 1127, 2451, 300101-3004, 3006-3013, 3015- 3022, 3103, 3104, 3204, 3402-3404, 3407, 3408, 3601, 3602
16 ⁹	180, 235 bp	1070 bp	01010102N, 0115N	
17	210 bp	1070 bp	0116N	
18 ⁵	135 bp	1070 bp	01010101-0104N, 0106, 0108-0112, 0114-0127N	3601-3604
19	165 bp	1070 bp	0118N	
20 ^{5,10}	60, 425 bp	800 bp	0110, 0119, 0121, 0126	
21	255 bp	1070 bp	0120	0219, 0236, 0237, 0254, 2414
22	600 bp	1070 bp	0122N	
23	155 bp	1070 bp	0124	
24 ⁶	215 bp	1070 bp	0112, 0119, 0125	0302, 0310, 110101-1107, 1109-1122, 1127- 1130, 1132, 2451

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*01 SSP subtypings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 bp or more. Size differences shorter than 20 bp are not given. For high resolution SSP kits the length of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

PCR fragments longer than the control 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.

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 tubes, or a band of 800 base pairs, for some tubes.

Tube number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-A*01 subtyping. .

In addition, tubes number 3, 7, 10 to 12 and 20 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

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.

³The nucleotide sequence of the A*0128 allele is not yet retrievable.

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

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

⁵Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁶Primer mixes 5, 15 and 24 may give rise to non-specific amplifications.

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

⁸Primer mix 12 might faintly amplify the A*0227, A*110101-110202, 1104-1107, 1109-1113, 1115-1119 and 1121N-1132 alleles.

⁹Primer mix 16: Specific PCR fragment of 180 bp in the A*01010102N allele. Specific PCR fragment of 235 bp in the A*0115N allele.

¹⁰Primer mix 20: Specific PCR fragment of 60 bp in the A*0110, A*0121 and A*0126 alleles. Specific PCR fragment of 425 bp in the A*0119 allele.

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INTERPRETATION TABLE												
HLA-A*01 SSP subtyping												
Amplification patterns of the A*0101 to 0127N alleles												
	Tube⁵											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	235	145	120	305	470	210	215	110	235	155	275	85
PCR product												
Length of int.	800	1070	800	1070	1070	1070	800	1070	1070	800	800	800
pos. control¹												
5'-primer(s)²	363	98	123	341	3rd I	171	363	203	363	413	363	527
	5'-ATA ^{3'}	5'-CTT ^{3'}	5'-AgT ^{3'}	5'-ggA ^{3'}	5'-ATA ^{3'}	5'-TTA ^{3'}	5'-ATA ^{3'}	5'-gAA ^{3'}	5'-ATA ^{3'}	5'-CCg ^{3'}	5'-ATA ^{3'}	5'-TgC ^{3'}
3'-primer(s)³	559	203	203	362	621	341	539	270	553	527	597	570
	5'-CCg ^{3'}	5'-TCT ^{3'}	5'-TCT ^{3'}	5'-TCA ^{3'}	5'-CCC ^{3'}	5'-CgT ^{3'}	5'-TCA ^{3'}	5'-ACA ^{3'}	5'-CTA ^{3'}	5'-CCC ^{3'}	5'-TTA ^{3'}	5'-CAC ^{3'}
									559			
									5'-CCA ^{3'}			
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-A allele⁴												
*01010101, 010102-010104	+	+										
*01010102N	+	+										
*0102	+		+									
*0103		+		+								
*0104N	+	+			+							
*0106	+	+					+					
*0107	+	+						+				
*0108		+							+			
*0109	+	+				+						
*0110	+	+								+		
*0111N	+	+									+	
*0112		+										
*0113	+	+										
*0114		+										
*0115N	+	+										
*0116N	+	+										
*0117	+	+										
*0118N	+	+										
*0119		+										
*0120			+									
*0121	+	+										
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE												
HLA-A*01 SSP subtyping												
Amplification patterns of the A*0101 to 0127N alleles												
Tube⁵												
13	14	15	16	17	18	19	20	21	22	23	24	
205	120	235	180	210	135	165	65	255	600	155	215	Length of spec.
			235				425					PCR product
1070	1070	1070	1070	1070	1070	1070	800	1070	1070	1070	1070	Length of int.
												pos. control ¹
363	203	363	203	363	203	215	203	355	3 rd I	363	363	5'-primer(s) ²
5'-ATA ^{3'}	5'-gAA ^{3'}	5'-ATA ^{3'}	5'-gAA ^{3'}	5'-ATA ^{3'}	5'-gAA ^{3'}	5'-gCC ^{3'}	5'-gAA ^{3'}	5'-CCg ^{3'}	5'-ATA ^{3'}	5'-ATA ^{3'}	5'-ATA ^{3'}	
			363				521					
			5'-ATA ^{3'}				5'-ggC ^{3'}					
527	282	559	341	531	299	341	346	570	750	203	538	3'-primer(s) ³
5'-CCA ^{3'}	5'-gAC ^{3'}	5'-CgT ^{3'}	5'-CgT ^{3'}	5'-TCC ^{3'}	5'-TCg ^{3'}	5'-CgT ^{3'}	5'-AgC ^{3'}	5'-CCg ^{3'}	5'-T.g ^{3'}	5'-TCT ^{3'}	5'-CTg ^{3'}	
			557				546					
			5'-gC ^{3'}				5'-AgA ^{3'}					
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.
												HLA-A allele ⁴
					+							*01010101, 010102-010104
			+		+							*01010102N
					+							*0102
					+							*0103
					+							*0104N
					+							*0106
					+							*0107
					+							*0108
					+							*0109
					+		+					*0110
					+							*0111N
+		+			+						+	*0112
	+											*0113
		+			+							*0114
			+		+							*0115N
				+	+							*0116N
	+				+							*0117
					+	+						*0118N
+		+			+		+				+	*0119
					+			+				*0120
+					+		+					*0121
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.

Lot No.: **X98**

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Length of spec.	235	145	120	305	470	210	215	110	235	155	275	85
PCR product												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
*0122N	+	+										
*0123	+	+						+				
*0124	+	+										
*0125	+	+										
*0126	+	+										
*0127N	+	+							+			
*0219, 0236, 0237, 0254, 2414												
*03010101-030105, 0303N, 0304, 0306, 0308, 0309, 0311N- 0317, 0319, 0320, 0322- 0330, 3204												
*0302, 0310, 2451												
*0305, 300101-3003, 3007, 3008, 3010-3013, 3015, 3016, 3018-3022, 3402-3404, 3407, 3408							+					
*0307, 3004, 3006, 3017												
*0318, 1108, 1123	+											
*0321N					+							
*110101-1103, 1105- 1107, 1109-1113, 1115- 1120, 1122, 1128-1130, 1132	+											
*1104, 1127												
*1114	+									W		
*1121N	+				+							
*1124, 1125	+						+					
*1126, 2632, 3313, 7410				+								
*1131	+						+					
*2307N, 2411N					+							
*3009, 3103, 3104							+					
*3014L							+					
*3601, 3602		+										+
*3603		+		+								+
*3604	+	+										+
*8001							+			W		
HLA-A allele ⁴												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12

HLA-A*01
 101.411-24/06 – licensed for PCR
 101.411-24u/06u – not licensed for PCR
 Lot No.: **X98**

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205	120	235	180	210	135	165	65	255	600	155	215	Length of spec. PCR product Tube No.
13	14	15	16	17	18	19	20	21	22	23	24	
					+				+			*0122N
					+							*0123
					+					+		*0124
					+						+	*0125
					+		+					*0126
					+							*0127N
								+				*0219, 0236, 0237, 0254, 2414
		+										*03010101-030105, 0303N, 0304, 0306, 0308, 0309, 0311N- 0317, 0319, 0320, 0322- 0330, 3204
+		+									+	*0302, 0310, 2451
		+										*0305, 300101-3003, 3007, 3008, 3010-3013, 3015, 3016, 3018-3022, 3402-3404, 3407, 3408
W		+										*0307, 3004, 3006, 3017 *0318, 1108, 1123
		+										*0321N
											+	*110101-1103, 1105- 1107, 1109-1113, 1115- 1120, 1122, 1128-1130, 1132
		+									+	*1104, 1127
											+	*1114
											+	*1121N
												*1124, 1125
												*1126, 2632, 3313, 7410
W												*1131
												*2307N, 2411N
W		+										*3009, 3103, 3104
												*3014L
		+			+							*3601, 3602
					+							*3603
					+							*3604
												*8001
												HLA-A allele ⁴
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.

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*01 subtyping. . In addition, tubes number 3, 7, 10 to 12 and 20 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

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.

²The nucleotide position, in the 2nd, 3rd or 4th exon or the 3rd 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.

³The nucleotide position, in 2nd, 3rd or 4th 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*0105N allele has been shown to be identical to A*0104N.

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

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

⁵Primer mix 16: Specific PCR fragment of 180 bp in the A*01010102N allele. Specific PCR fragment of 235 bp in the A*0115N allele.

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

'w', might be faintly amplified.

CELL LINE VALIDATION SHEET																			
HLA-A*01 SSP subtyping kit																			
			Tube																
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
			Prod. No.:	200622901	200735502	200622903	200622904	200622905	200735506	200622907	200735508	200735509	200622910	200622911	200622912	200735513	200622914	200622915	200735516
	cell line	A*																	
1	9001 SA	*2402	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2	9280 LK707	*0201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
3	9011 E4181324	*0101	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4	9275 GU373	*3001	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	
5	9009 KAS011	*0101	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
6	9353 SM	*0201	*2603	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
7	9020 QBL	*2601	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
8	9007 DEM	*0201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
9	9026 YAR	*2601	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
10	9107 LKT3	*2402	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
11	9051 PITOUT	*2902	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
12	9052 DBB	*0201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
13	9067 BTB	*0201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
14	9071 OLGA	*3101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
15	9075 DKB	*2402	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
16	9037 SWEIG007	*2902	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
17	9008 WILJON	*2501	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
18	9257 32367	*3303	*7401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
19	9038 BM16	*0201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
20	9059 SLE005	*0201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
21	9064 AMALA	*0217	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
22	9056 KOSE	*0201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
23	9124 IHL	*0201	*3401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
24	9035 JBUSH	*3201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
25	9049 IBW9	*3301	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
26	9285 WT49	*0205	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
27	9191 CH1007	*2410	*2901	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
28	9320 BEL5GB	*0201	*2902	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
29	9050 MOU	*2902	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
30	9021 RSH	*3001	*6802	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	
31	9019 DUCAF	*3002	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	
32	9297 HAG	*0201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
33	9098 MT14B	*3101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
34	9104 DHIF	*3101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
35	9302 SSTO	*3201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
36	9024 KT17	*0206	*1101	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
37	9065 HHKB	*0301	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	
38	9099 LZL	*0217	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
39	9315 CML	*0101	*0301	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	
40	9134 WHONP199	*0207	*3001	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	
41	9055 H0301	*0301	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	
42	9066 TAB089	*0207	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
43	9076 T7526	*0207	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
44	9057 TEM	*6601	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
45	9239 SHJO	*2301	*2402	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
46	9013 SCHU	*0301	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	
47	9045 TUBO	*0216	*0301	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	
48	9303 TER-ND	*0201	*1101	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
49	9366 DAUDI	*0102	*6601	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	

CELL LINE VALIDATION SHEET												
HLA-A*01 SSP subtyping kit												
				Tube								
				17	18	19	20	21	22	23	24	
				Prod. No.:	200622917	200622918	200622919	200735520	200735521	200735522	200735523	200735524
cell line			A*									
1	9001	SA	*2402	-	-	-	-	-	-	-	-	-
2	9280	LK707	*0201	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*0101	-	+	-	-	-	-	-	-	-
4	9275	GU373	*3001	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*0101	-	+	-	-	-	-	-	-	-
6	9353	SM	*0201	*2603	-	-	-	-	-	-	-	-
7	9020	QBL	*2601	-	-	-	-	-	-	-	-	-
8	9007	DEM	*0201	-	-	-	-	-	-	-	-	-
9	9026	YAR	*2601	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*2402	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*2902	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0201	-	-	-	-	-	-	-	-	-
13	9067	BTB	*0201	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*3101	-	-	-	-	-	-	-	-	-
15	9075	DKB	*2402	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*2902	-	-	-	-	-	-	-	-	-
17	9008	WILJON	*2501	-	-	-	-	-	-	-	-	-
18	9257	32367	*3303	*7401	-	-	-	-	-	-	-	-
19	9038	BM16	*0201	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*0201	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*0217	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*0201	-	-	-	-	-	-	-	-	-
23	9124	IHL	*0201	*3401	-	-	-	-	-	-	-	-
24	9035	JBUSH	*3201	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*3301	-	-	-	-	-	-	-	-	-
26	9285	WT49	*0205	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*2410	*2901	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*0201	*2902	-	-	-	-	-	-	-	-
29	9050	MOU	*2902	-	-	-	-	-	-	-	-	-
30	9021	RSH	*3001	*6802	-	-	-	-	-	-	-	-
31	9019	DUCAF	*3002	-	-	-	-	-	-	-	-	-
32	9297	HAG	*0201	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*3101	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*3101	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*3201	-	-	-	-	-	-	-	-	-
36	9024	KT17	*0206	*1101	-	-	-	-	-	-	-	+
37	9065	HHKB	*0301	-	-	-	-	-	-	-	-	-
38	9099	LZL	*0217	-	-	-	-	-	-	-	-	-
39	9315	CML	*0101	*0301	-	+	-	-	-	-	-	-
40	9134	WHONP199	*0207	*3001	-	-	-	-	-	-	-	-
41	9055	H0301	*0301	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*0207	-	-	-	-	-	-	-	-	-
43	9076	T7526	*0207	-	-	-	-	-	-	-	-	-
44	9057	TEM	*6601	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*2301	*2402	-	-	-	-	-	-	-	-
46	9013	SCHU	*0301	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*0216	*0301	-	-	-	-	-	-	-	-
48	9303	TER-ND	*0201	*1101	-	-	-	-	-	-	-	+
49	9366	DAUDI	*0102	*6601	-	+	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP™ HLA-A*01 SSP

Product number: 101.411-24/06 – licensed for PCR
101.411-24u/06u – not licensed for PCR

Lot number: X98

Expiry date: 2009-June-01

Number of tests: 24 test – Product No. 101.411-24
6 tests – Product No. 101.411-06

Number of tubes per test: 24

Tube specifications:

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

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

No DNAs carrying the alleles to be amplified by primer solutions 4, 6, 8, 9, 10, 11, 14, 17, 19 to 23 were available. The specificities of primers in primer solutions 4, 8, 14, 21 and 23 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 6 and 19 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 9, 10, 11, 17, 20 and 22 it was only possible to test the 5'-primers, the 3'-primers were not possible to test.

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

Date of approval: 2007-August-21

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: Olerup SSP™ HLA-A*01
Product number: 101.411-24/06, 101.411-24u/06u
Lot number: X98

Intended use: HLA-A*01 high resolution histocompatibility testing

Manufacturer: Olerup SSP AB
Hasselstigen 1
SE-133 33 Saltsjöbaden, Sweden
Phone: +46-8-717 88 27
Fax: +46-8-717 88 18

We, Olerup SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2000 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at Olerup SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

The Authorized Representative located within the Community is: Olerup SSP AB.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom. (Notified Body number: 0088.)

Saltsjöbaden, Sweden
2007-August-21

Olle Olerup
Managing Director

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.

Olerup SSPTM is a trademark of *Olerup* SSP AB.
PCRTM is a trademark of F. Hoffmann-La Roche Ltd.
ARMSTM is a trademark of Zeneca Ltd.

HLA-A*01
101.411-24/06 – licensed for PCR
101.411-24u/06u – not licensed for PCR
Lot No.: **X98**

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