

Olerup SSP™ HLA-A*68

Product number: 101.418-12/04 – licensed for PCR
101.418-12u/04u – not licensed for PCR
Lot number: X65
Expiry date: 2009-May-01
Number of tests: 12 tests – Product No. 101.418-12
4 tests – Product No. 101.418-04
Number of tubes per test: 32
Storage - pre-aliquoted primers: dark at -20°C
- PCR Master Mix: -20°C

This Product Description is only valid for Lot No. X65.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP™ HLA-A*68 LOT

The HLA-A*68 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous Olerup SSP™ HLA-A*68 lot was made (Lot No. V53).

Eight tubes have been added to the HLA-A*68 kit,
wells **25 to 32**.

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

Tube	5'-primer	3'-primer	rationale
1	-	Modified	Increased resolution, no amplification of A*29 alleles.
2	-	Modified	Increased resolution, no amplification of A*33 alleles.
3	Removed	-	A*6829 primer moved to vial 25.
4	Modified	-	Increased specificity of specific primers.
6	Modified	-	Increased specificity of specific primers.
9	-	Modified	Increased specificity of specific primers.
18	Modified	-	Increased specificity of specific primers.
22	-	Modified	Increased specificity of specific primers.
25	New	New	Primer pair for the A*6829 allele from vial 3.
26	New	New	Primer pair for the A*6833 allele.

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27	New	New	Primer pair for the A*6834 allele.
28	New	New	Primer pair for the A*6835 allele.
29	New	New	Primer pair for the A*6836 allele, also primer for distinguish A*6827 from A*34xx and A*66xx.
30	New	New	Primer pair for the A*6837 allele.
31	New	New	Primer pair for the A*6838 allele.
32	New	New	Primer pair for distinguishing the A*01xx,6801 and A*01xx,6821 genotypes.

PRODUCT DESCRIPTION

HLA-A*68 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*6801 to A*6838 alleles. The HLA-A*6901 allele is also identified.

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.

32 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 32 PCR reactions in a 32 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
25	26	27	28	29	30	31	32

The 32 well cut PCR plate is marked with 'A*68, X65'.

Well No. 1 is marked with '1'.

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

Please note: When removing each 32 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*68 SSP subtypings will be influenced by most A*01 alleles, several A*02 alleles, most A*03 alleles, all A*11 alleles, one A*23, five A*24 alleles, most A*25, the A*26, most A*29 alleles, one A*30 allele, three A*31 alleles, two A*32 allele, all A*34 alleles, all A*36, the A*4301, the A*66 alleles and the A*8001 allele as well as by the A*6901 allele when present on the other haplotype.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A28 alleles¹, i.e. **A*6801 to A*6838 as well as the A*6901 allele**, recognized by the HLA Nomenclature Committee in April 2007² will give rise to unique amplification patterns by the primers in the HLA-A*68 subtyping kit.

The HLA-A*68 subtyping kit cannot distinguish the A*680101 to A*680106 alleles, the A*68020101 and A*68020102 alleles and the A*680301 and A*680302 alleles.

¹The nucleotide sequences of the A*68020103 and A*6839 alleles are 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-April-12, release 2.17.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 38 HLA-A*68 alleles can be combined in 741 homozygous and heterozygous combinations. 271 of these genotypes do not give rise to unique amplification patterns. The different lengths of specific PCR fragments yielded by primer mixes 4, 5, 7, 11, 15, 16, 17, 18, 21 and 30 were not considered in these calculations.

+++++---	+-----	+-----+	-----	6805,6818N = 6818N,6820
+++++---	-----	+-----+	-----	6805,6828 = 6820,6828
+++++---	-----	+-----+	--+-----	6805,6834 = 6820,6834
+++++---	-----	+-----+	-----	6802,6805 = 6802,6820
+++++---	+-----	+-----+	-----	6804,6818N = 6818N,6825
+++++---	-----	+-----+	-----	6804,6828 = 6825,6828
+++++---	-----	+-----+	--+-----	6804,6834 = 6825,6834
+++++---	-----	+-----+	-----	6802,6804 = 6802,6825
+++--+-	-----+	+-----+	-----	6801,6815 = 6815,6816
+++-----	+-----	+-----+	-----	6802,6808 = 6808,6818N
+++-----	-----+	+-----+	-----	6816,6828 = 6824,6831
+++-----	-----+	+-----+	-----	6801,6831 = 6802,6816 = 6816,6831
+++-----	-----	+-----+	-----	6801,6828 = 6802,6824 = 6824,6828
++-++---	-----	-----+	-----	6803,6825 = 6804,6830 = 6825,6830
++-++++-	-----	+-----+	-----	6806,6820 = 6820,6832
++-++++-	-----	-----+	-----	6805,6806 = 6805,6832
++-++++-	--+-----	+-----+	-----	6810,6820 = 6817,6820
++-++++-	--+-----	-----+	-----	6805,6810 = 6805,6817
++-++++-	-----	+-----+	-----	6801,6820 = 6805,6827 = 6820,6827 = 6820,6830
++-++---	-----	-----+	-----	6801,6805 = 6805,6830
++-++---	-----	-----+	-----	6803,6806 = 6803,6832 = 6830,6832
++-++---	+-----	-----+	-----	6803,6808 = 6808,6830
++-++---	-+-----	-+-----+	-----	6803,6826 = 6826,6830
++-++---	-+-----	-----+	-----	6803,6809 = 6809,6830
++-++---	--+-----	-----+	-----	6803,6810 = 6803,6817 = 6817,6830
++-++---	---+-----	-----+	-----	6803,6811N = 6811N,6830
++-++---	-----+	-----+	-----	6803,6812 = 6812,6830
++-++---	-----+	-----+	-----	6803,6819 = 6819,6830
++-++---	-----+	-----+	-----	6803,6816 = 6816,6830
++-++---	-----	-+-----+	-----+	6803,6821 = 6821,6830
++-++---	-----	--+-----+	-----	6803,6822 = 6822,6830
++-++---	-----	-----+	-----	6803,6823 = 6823,6830
++-++---	-----	-----+	-----	6803,6824 = 6824,6830

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++-+-----	-----	-----++-	+-----	6803,6829 = 6829,6830
++-+-----	-----	-----++-	-+-----	6803,6833 = 6830,6833
++-+-----	-----	-----++-	---+-----	6803,6835 = 6830,6835
++-+-----	-----	-----++-	-----+--	6803,6837 = 6830,6837
++-+-----	-----	-----++-	-----+--	6803,6838 = 6830,6838
++-+-----	-----	-----++-	-----	6801,6803 = 6801,6830 = 6803,6830
++-+-----	-----	-----++-	-----	6804,6806 = 6804,6832 = 6806,6825 = 6825,6832
++-+-----	-----	-----++-	-----	6804,6807 = 6807,6825
++-+-----	+-----	-----++-	-----	6804,6808 = 6808,6825
++-+-----	-+-----	-+-----	-----	6804,6826 = 6825,6826
++-+-----	-+-----	-----++-	-----	6804,6809 = 6809,6825
++-+-----	---+-----	-----++-	-----	6804,6814 = 6814,6825
++-+-----	---+-----	-----++-	-----	6804,6810 = 6804,6817 = 6810,6825 = 6817,6825
++-+-----	---+-----	-----++-	-----	6804,6811N = 6811N,6825
++-+-----	-----+--	-----++-	-----	6804,6812 = 6812,6825
++-+-----	-----+--	-----++-	-----	6804,6813 = 6813,6825
++-+-----	-----+--	-----++-	-----	6804,6819 = 6819,6825
++-+-----	-----+--	-----++-	-----	6804,6816 = 6816,6825
++-+-----	-----+--	+-----++-	-----	6804,6827 = 6825,6827
++-+-----	-----	-+-----	-----+	6804,6821 = 6821,6825
++-+-----	-----	---+-----	-----	6804,6822 = 6822,6825
++-+-----	-----	---+-----	-----	6804,6823 = 6823,6825
++-+-----	-----	-----+++	-----	6804,6824 = 6824,6825
++-+-----	-----	-----++-	+-----	6804,6829 = 6825,6829
++-+-----	-----	-----++-	-+-----	6804,6833 = 6825,6833
++-+-----	-----	-----++-	---+-----	6804,6835 = 6825,6835
++-+-----	-----	-----++-	-----+--	6804,6837 = 6825,6837
++-+-----	-----	-----++-	-----+--	6804,6838 = 6825,6838
++-+-----	-----	-----++-	-----	6801,6804 = 6801,6825 = 6804,6825 = 6825,6825
++-+-----	---+-----	-----++-	-----	6806,6817 = 6810,6832 = 6817,6832
++-+-----	---+-----	-----++-	-----	6806,6811N = 6811N,6832
++-+-----	---+-----	-----++-	-----	6806,6812 = 6812,6832
++-+-----	-----+--	-----++-	-----	6806,6819 = 6819,6832
++-+-----	-----+--	-----++-	-----	6806,6816 = 6816,6832
++-+-----	-----	-+-----	-----+	6806,6821 = 6821,6832
++-+-----	-----	---+-----	-----	6806,6822 = 6822,6832
++-+-----	-----	---+-----	-----	6806,6823 = 6823,6832
++-+-----	-----	-----+++	-----	6806,6824 = 6824,6832
++-+-----	-----	-----++-	+-----	6806,6829 = 6829,6832
++-+-----	-----	-----++-	-+-----	6806,6833 = 6832,6833
++-+-----	-----	-----++-	---+-----	6806,6835 = 6832,6835
++-+-----	-----	-----++-	-----+--	6806,6836 = 6832,6836
++-+-----	-----	-----++-	-----+--	6806,6837 = 6832,6837
++-+-----	-----	-----++-	-----+--	6806,6838 = 6832,6838
++-+-----	-----	-----++-	-----	6801,6806 = 6801,6832 = 6806,6832 = 6832,6832
++-+-----	+-----	-----++-	-----	6808,6810 = 6808,6817
++-+-----	-+-----	-+-----	-----	6810,6826 = 6817,6826
++-+-----	-+-----	-----++-	-----	6809,6810 = 6809,6817
++-+-----	-+-----	-+-----	-----+	6809,6821 = 6821,6826

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++----- --++----- -----+- -----	6810,6811N = 6811N,6817
++----- --+--+----- -----+- -----	6810,6812 = 6812,6817
++----- --+-----+-----+- -----	6801,6814 = 6810,6819 = 6814,6817 = 6814,6819 = 6817,6819
++----- --+-----+-----+- -----	6810,6816 = 6816,6817
++----- --+----- -+-----+-----+-----	6810,6821 = 6817,6821
++----- --+----- -+-----+-----+-----	6810,6822 = 6817,6822
++----- --+----- -+-----+-----+-----	6810,6823 = 6817,6823
++----- --+----- -+-----+-----+-----	6810,6824 = 6817,6824
++----- --+----- -+-----+-----+-----	6810,6829 = 6817,6829
++----- --+----- -+-----+-----+-----	6810,6833 = 6817,6833
++----- --+----- -+-----+-----+-----	6810,6835 = 6817,6835
++----- --+----- -+-----+-----+-----	6810,6836 = 6817,6836
++----- --+----- -+-----+-----+-----	6810,6837 = 6817,6837
++----- --+----- -+-----+-----+-----	6810,6838 = 6817,6838
++----- --+----- -+-----+-----+-----	6801,6810 = 6801,6817 = 6810,6817 = 6817,6817
++----- --+----- -+-----+-----+-----	6801,6811N = 6811N,6811N
++----- --+----- -+-----+-----+-----	6801,6812 = 6812,6812
++----- --+----- -+-----+-----+-----	6801,6819 = 6819,6819
++----- --+----- -+-----+-----+-----	6801,6816 = 6816,6816
++----- --+----- -+-----+-----+-----	6801,6821 = 6821,6821
++----- --+----- -+-----+-----+-----	6801,6822 = 6822,6822
++----- --+----- -+-----+-----+-----	6801,6823 = 6823,6823
++----- --+----- -+-----+-----+-----	6801,6824 = 6824,6824
++----- --+----- -+-----+-----+-----	6801,6829 = 6829,6829
++----- --+----- -+-----+-----+-----	6801,6833 = 6833,6833
++----- --+----- -+-----+-----+-----	6801,6835 = 6835,6835
++----- --+----- -+-----+-----+-----	6801,6837 = 6837,6837
++----- --+----- -+-----+-----+-----	6801,6838 = 6838,6838
++----- --+----- -+-----+-----+-----	6802,6815 = 6815,6827
++----- --+----- -+-----+-----+-----	6802,6818N = 6818N,6818N = 6818N,6827
++----- --+----- -+-----+-----+-----	6802,6831 = 6827,6831
++----- --+----- -+-----+-----+-----	6802,6828 = 6827,6828 = 6828,6828
++----- --+----- -+-----+-----+-----	6802,6834 = 6827,6834 = 6834,6834
++----- --+----- -+-----+-----+-----	6802,6802 = 6802,6827
++----- --+----- -+-----+-----+-----	6810,6814 = 6814,6814
++----- --+----- -+-----+-----+-----	6803,6815 = 6805,6815 = 6805,6831 = 6815,6820 = 6820,6831
++----- --+----- -+-----+-----+-----	6803,6820 = 6805,6820 = 6820,6820
++----- --+----- -+-----+-----+-----	6803,6805 = 6805,6805
++----- --+----- -+-----+-----+-----	6815,6815 = 6815,6831

LICENSES

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101.418-12/04 and 101.418-12u/04u

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*68 subtyping 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

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For one HLA-A*68 subtyping, add at room temperature in a 0.5 ml tube:

38 x 2 μl = 76 μl DNA (30 ng/μl)

38 x 3 μl = 114 μl PCR Master Mix complete with *Taq* – mix well before taking your aliquot

38 x 5 μl = 190 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-H₂O mixture into each of the 32 tubes of an HLA-A*68 subtyping. **Well No. 1 of the 32 well PCR plate is marked with '1'**. Close the 32 well PCR plate with the provided lids.

101.418-12u/04u – not licensed for PCR

For one HLA-A*68 subtyping, add at room temperature in a 0.5 ml tube:

38 x 2 μl = 76 μl DNA (30 ng/μl)

38 x 3 μl = 114 μl PCR Master Mix without *Taq* – mix well before taking your aliquot

3.0 μl *Taq* polymerase (5 units/μl)

38 x 5 μl – 3.0 μl = 187 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-*Taq*-H₂O mixture into each of the 32 tubes of an HLA-A*68 subtyping. **Well No. 1 of the 32 well PCR plate is marked with '1'**. Close the 32 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.

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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*68 SSP subtyping

Specificities and sizes of the PCR products of the 32 primer mixes used for HLA-A*68 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*68 alleles	Other amplified HLA-A alleles ³
1⁴	195 bp	800 bp	680101-68020102, 6806-6814, 6816-6819, 6821-6830, 6832-6835, 6837, 6838	0113, 0234-023502, 0256, 0262, 0278, 9203, 03010101-0307, 0309-0329, 110101-110107, 1103-1113, 1115, 1120-1130, 2419, 3018, 3401-3408, 6601, 6602, 6604, 6606, 6901
2	435 bp	1070 bp	680101-680106, 680301-6805, 6808, 6809, 6811N, 6812, 6816, 6817, 6819-6826, 6829, 6832, 6833, 6835-6838	0324, 1110, 3402-3404, 3407, 3408
3	195 bp	1070 bp	68020101-68020102, 6815, 6818N, 6828, 6831, 6834	
4⁵	180, 220 bp	1070 bp	680301-680302, 6805, 6820, 6830	
5⁶	140, 235 bp	800 bp	6804, 6825	
6	225 bp	1070 bp	6805, 6815, 6820	
7⁷	135, 155 bp	1070 bp	6806, 6832	0252
8	160 bp	1070 bp	6807	
9	160 bp	1070 bp	6808, 6818N	030102, 1124, 8001
10	165 bp	800 bp	6809, 6826	0112, 0119, 0310, 110101-1107, 1109 ^{weakly} , 1110-1121N, 1126-1130, 2417, 2441, 2905
11⁸	160, 200 bp	800 bp	6810, 6814, 6817	
12	405 bp	1070 bp	6811N	
13	215 bp	1070 bp	6812	3601-3604 ^{faintly}
14	195 bp	1070 bp	6813	

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15^{9,10}	145, 205 bp	1070 bp	6812 [?] , 6814, 6819	0317, 2418
16¹¹	150, 215 bp	1070 bp	6815, 6816, 6831	
17¹²	165, 185 bp	1070 bp	68020101- 68020102, 6815, 6818N, 6820, 6827, 6828, 6831, 6834	0202 ^{weakly} , 020301-020302, 0205 ^{weakly} , 0208 ^{weakly} , 0222, 0247 ^{weakly} , 0249, 0250, 0263 ^{weakly} , 9202 ^{weakly} , 9204, 9215 ^{weakly} , 2302, 2406, 250101-2503, 2505, 2506, 260101-260104, 2602- 260702, 2609-2626, 2628- 2634, 3210, 3401, 3405, 4301, 6601-6605
18¹³	195, 225 bp	1070 bp	6821, 6826	01010101-0104, 0106-0125, 0304, 0318, 1127, 2417, 2441, 2903, 8001
19⁹	325 bp	1070 bp	6822	
20^{9,14}	90 bp	1070 bp	6823	0120, 9214
21¹⁵	165, 215 bp	1070 bp	6824, 6828	0249
22⁴	165 bp	800 bp	680101-680106, 680301-6805, 6807, 6810-6814, 6816, 6817, 6819-6825, 6829, 6830, 6832, 6835-6838	01010101-0104N ^{weakly} , 0107- 0111N ^{weakly} , 0113- 0118N ^{weakly} , 0120-0124 ^{weakly} , 0318 ^{weakly} , 1108, 3601- 3604 ^{weakly}
23	185 bp	1070 bp	680101-680106, 680301-6805, 6807- 6814, 6816, 6817, 6819-6825, 6829, 6830, 6832, 6832, 6835-6838	9214, 03010101-0317, 0320- 0329, 1104, 1127, 29010101- 290201, 290203, 2904-2916, 3103, 3104, 3106, 3204, 3402-3404, 3407, 3408, 3601-3603
24¹⁶	375 bp	1070 bp		0255, 2622, 6901
25	240 bp	1070 bp	6829	
26	185 bp	1070 bp	6833	
27	190 bp	1070 bp	6834	2604
28	200 bp	1070 bp	6835	
29	255 bp	800 bp	6836	
30¹⁷	145, 245 bp	1070 bp	6837	250101-2506, 260101-2610, 2612, 2613, 2615-2617, 2619-2627, 2629-2634, 3401, 3406, 4301, 6601-6606
31	205 bp	1070 bp	6838	
32	225 bp	1070 bp	6821	0304

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*68 SSP subtypings.

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

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

PCR fragments longer than the control band may sometimes be observed. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

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*68 subtyping.

In addition, tubes number 5, 10, 11, 22 and 29 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 sequences of the A*68020103 and A*6839 alleles are not yet retrievable.

Due to the sharing of sequence motifs between HLA-A alleles non-HLA-A*68 alleles will be amplified by primer mixes 1, 2, 7, 9, 10, 13, 15, 17, 18, 21, 20, 22, 23, 24, 27 and 30.

⁴Primer mixes 1 and 22 may yield less specific PCR product than the other A*68 primer mixes.

⁵Primer mix 4: Specific PCR fragment of 180 bp in the A*6830 allele. Specific PCR fragment of 220 bp in the A*680301-680302, A*6805 and A*6820 alleles.

⁶Primer mix 5: Specific PCR fragment of 140 bp in the A*6825 allele. Specific PCR fragment of 235 bp in the A*6804 allele.

⁷Primer mix 7: Specific PCR fragment of 135 bp in the A*6806 and A*0252 alleles. Specific PCR fragment of 155 bp in the A*6832 allele.

⁸Primer mix 11: Specific PCR fragment of 160 bp in the A*6817 allele. Specific PCR fragment of 200 bp in the A*6810 and A*6814 alleles.

⁹Primer mixes 15, 19 and 20 have a tendency of giving rise to a primer oligomer artifact.

¹⁰Primer mix 15: Specific PCR fragment of 145 bp in the A*6819 and A*0317, A*2418 alleles. Specific PCR fragment of 205 bp in the A*6814 allele.

¹¹Primer mix 16: Specific PCR fragment of 150 bp in the A*6816 allele. Specific PCR fragment of 215 bp in the A*6815 and A*6831 alleles.

¹²Primer mix 17: Specific PCR fragment of 165 bp in the A*68020101-68020102, A*6815, A*6818N, A*6827, A*6828, A*6831, A*6834 and A*0202, A*020301-020302, A*0205, A*0208, A*0222, A*0247, A*0249, A*0250, A*0263, A*9202, A*9204, A*9215, A*2302, A*2406, A*250101-2503, A*2505, A*2506, A*260101-260104, A*2602-260702, A*2609-2626, A*2628-2634, A*3210, A*3401, A*3405, A*4301, A*6601 to A*6605 alleles. Specific PCR fragment of 185 bp in the A*6820 allele.

¹³Primer mix 18: Specific PCR fragment of 195 bp in the A*6826 and A*01010101-0104N, A*0106-0125, A*0318, A*1127A*2417, A*2441, A*2903 and A*8001 alleles. Specific PCR fragment of 225 bp in the A*6821 and A*0304 alleles.

¹⁴Short specific PCR fragments are less intense and not as sharp as longer specific bands.

¹⁵Primer mix 21: Specific PCR fragment of 165 bp in the A*6828 and A*0249 alleles. Specific PCR fragment of 215 bp in the A*6824 allele.

¹⁶Primer mix 24 may give to non-specific amplifications.

¹⁷Primer mix 30: Specific PCR fragment of 145 bp in the A*250101-2506, A*260101-2610, A*2612, A*2613, A*2615-2617, A*2619-2627, A*2629-2634, A*3401, A*3406, A*4301, A*6601-6606 alleles. Specific PCR fragment of 245 bp in the A*6837 allele.

'?', nucleotide sequence information not available for the primer matching sequence.

INTERPRETATION TABLE																
HLA-A*68 SSP subtyping																
Amplification patterns of the A*6801 to A*6838 alleles																
	Tube⁵															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length of spec.	195	435	195	180	140	225	135	160	160	165	160	405	215	195	145	150
PCR product				220	235		155				200				205	215
Length of int.	800	1070	1070	1070	800	1070	1070	1070	1070	800	800	1070	1070	1070	1070	1070
pos. control¹																
5'-primer(s)²	127	261	106	102	98	102	145	418	106	413	98	47	2 nd I	103	453	106
	5'-ggg ^{3'}	5'-AAC ^{3'}	5'-CCA ^{3'}	5'-ACT ^{3'}	5'-CTA ^{3'}	5'-ACT ^{3'}	5'-CCA ^{3'}	5'-AgC ^{3'}	5'-CCA ^{3'}	5'-CCg ^{3'}	5'-CTA ^{3'}	5'-g.T ^{3'}	5'-CCT ^{3'}	5'-CCT ^{3'}	5'-AAA ^{3'}	5'-CCA ^{3'}
					397	106	414		413		419					413
					5'-gCC ^{3'}	5'-CCA ^{3'}	5'-gAA ^{3'}		5'-CCg ^{3'}		5'-ggT ^{3'}					5'-CCg ^{3'}
3'-primer(s)³	282	413	259	240	290	292	259	539	216	538	256	282	497	256	555	282
	5'-gAC ^{3'}	5'-gCC ^{3'}	5'-gTT ^{3'}	5'-ggA ^{3'}	5'-CAA ^{3'}	5'-gTg ^{3'}	5'-gTT ^{3'}	5'-TCC ^{3'}	5'-gCT ^{3'}	5'-CTg ^{3'}	5'-CTC ^{3'}	5'-gAC ^{3'}	5'-TgA ^{3'}	5'-Cgg ^{3'}	5'-CCA ^{3'}	5'-gAg ^{3'}
		413		282	497		506		538		538				616	524
		5'-gCT ^{3'}		5'-gAg ^{3'}	5'-Tgg ^{3'}		5'-TgT ^{3'}		5'-CAg ^{3'}		5'-CCA ^{3'}				5'-CgC ^{3'}	5'-CAA ^{3'}
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
HLA-A allele⁴																
*680101-680106	+	+														
*68020101-68020101	+		+													
*680301-680302		+		+												
*6804		+			+											
*6805		+		+		+										
*6806	+						+									
*6807	+							+								
*6808	+	+							+							
*6809	+	+								+						
*6810	+										+					
*6811N	+	+										+				
*6812	+	+											+		?	
*6813	+													+		
*6814	+										+				+	
*6815			+			+										+
*6816	+	+														+
*6817	+	+									+					
*6818N	+		+						+							
*6819	+	+													+	
*6820		+		+		+										
*6821	+	+														
*6822	+	+														
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

INTERPRETATION TABLE																
HLA-A*68 SSP subtyping																
Amplification patterns of the A*6801 to A*6838 alleles																
Tube⁵																
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
165	195	325	90	165	165	185	375	240	185	190	200	255	145	205	225	Length of spec.
185	225			215									245			PCR product
1070	1070	1070	1070	1070	800	1070	1070	1070	1070	1070	1070	800	1070	1070	1070	Length of int.
																pos. control ¹
395	413	317	362	102	413	413	261	28	666	413	121	102	102	376	413	5'-primer(s) ²
5'-gCA ^{3'}	5'-CCg ^{3'}	5'-gCA ^{3'}	5'-gAg ^{3'}	5'-ACT ^{3'}	5'-CCg ^{3'}	5'-CCg ^{3'}	5'-AAC ^{3'}	5'-TCg ^{3'}	5'-ggA ^{3'}	5'-CCA ^{3'}	5'-gCT ^{3'}	5'-ACT ^{3'}	5'-ACT ^{3'}	5'-gCA ^{3'}	5'-CCg ^{3'}	
413				413									414			
5'-CCA ^{3'}				5'-CCA ^{3'}									5'-CAg ^{3'}			
538	570	362	413	275	539	559	355	97	808	560	282	317	308	538	595	3'-primer(s) ³
5'-CCA ^{3'}	5'-CCg ^{3'}	5'-TCA ^{3'}	5'-gCC ^{3'}	5'-CCC ^{3'}	5'-TCC ^{3'}	5'-CgT ^{3'}	5'-gAC ^{3'}	5'-ggT ^{3'}	5'-AgA ^{3'}	5'-ACA ^{3'}	5'-gAC ^{3'}	5'-ggA ^{3'}	5'-TCT ^{3'}	5'-CCA ^{3'}	5'-CCg ^{3'}	
	595			538									517			
	5'-CCg ^{3'}			5'-CCg ^{3'}									5'-CgT ^{3'}			
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.
																HLA-A allele ⁴
					+	+										*680101-680106
+					+	+										*68020101-68020101
					+	+										*680301-680302
					+	+										*6804
					+	+										*6805
					+	+										*6806
					+	+										*6807
						+										*6808
						+										*6809
					+	+										*6810
					+	+										*6811N
					+	+										*6812
					+	+										*6813
					+	+										*6814
+					+	+										*6815
					+	+										*6816
					+	+										*6817
+					+	+										*6818N
					+	+										*6819
+					+	+										*6820
	+				+	+									+	*6821
		+			+	+										*6822
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.

Lot No.: **X65**

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Length of spec.	195	435	195	180	140	225	135	160	160	165	160	405	215	195	145	150
PCR product				220	235		155				200				205	215
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*6823	+	+														
*6824	+	+														
*6825	+	+			+											
*6826	+	+								+						
*6827	+															
*6828	+		+													
*6829	+	+														
*6830	+			+												
*6831			+													+
*6832	+	+					+									
*6833	+	+														
*6834	+		+													
*6835	+	+														
*6836		+														
*6837	+	+														
*6838	+	+														
*01010101-0104N, 0107-0111N, 0114- 0118N, 0121-0124																
*0106, 01252903																
*0112, 0119										+						
*0113, 0318	+															
*0120																
*0202, 0205, 0208, 0247, 0263, 9202, 9215																
*020301-020302, 0222, 0250, 9204, 2302, 2406, 2611N, 2614, 2618, 2628, 3210																
*0234-023502, 0256, 0262, 0278, 9203, 0319, 1122, 1123, 1125, 2419, 3018, 3406, 6606	+															
*0249																
*0252							+									
*0255																
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

165	195	325	90	165	165	185	375	240	185	190	200	255	145	205	225	Length of spec.
185	225			215									245			PCR product
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.
			+		+	+										*6823
				+	+	+										*6824
					+	+										*6825
	+															*6826
+																*6827
+				+												*6828
					+	+		+								*6829
					+	+										*6830
+																*6831
					+	+										*6832
					+	+			+							*6833
+										+						*6834
					+	+					+					*6835
					+	+						+				*6836
					+	+							+			*6837
					+	+								+		*6838
	+				W											*01010101-0104N, 0107-0111N, 0114- 0118N, 0121-0124
	+															*0106, 01252903
	+															*0112, 0119
	+				W											*0113, 0318
	+		+		W											*0120
W																*0202, 0205, 0208, 0247, 0263, 9202, 9215
+																*020301-020302, 0222, 0250, 9204, 2302, 2406, 2611N, 2614, 2618, 2628, 3210
																*0234-023502, 0256, 0262, 0278, 9203, 0319, 1122, 1123, 1125, 2419, 3018, 3406, 6606
+				+												*0249
																*0252
							+									*0255
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.

Length of spec.	195	435	195	180	140	225	135	160	160	165	160	405	215	195	145	150
PCR product				220	235		155				200				205	215
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*9214																
*03010101-03010103, 030103-0303N, 0305-0307, 0309, 0311N-0316, 0320-0323, 0325-0329	+															
*030102	+								+							
*0304	+															
*0308, 29010101-290201, 290203, 2904, 2906-2916, 3103, 3104, 3106, 3204																
*0310, 1104	+									+						
*0317	+														+	
*0324	+	+														
*110101-110107, 1103, 1105-1107, 1111-1113, 1115, 1120, 1121N, 1126, 1128-1130	+									+						
*110201-110202, 1114, 1116-1119										+						
*1108	+															
*1109	+									W						
*1110	+	+								+						
*1124	+								+							
*1127	+									+						
*2417, 2441										+						
*2418															+	
*250101-2503, 2505, 2506, 260101-260104, 2602, 2603, 2605-260702, 2609, 2610, 2612, 2613, 2615-2617, 2619-2621, 2623-2626, 2629-2634, 4301, 6603, 6605																
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

165	195	325	90	165	165	185	375	240	185	190	200	255	145	205	225	Length of spec.
185	225			215									245			PCR product
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.
			+			+										*9214
						+										*03010101-03010103, 030103-0303N, 0305-0307, 0309, 0311N-0316, 0320-0323, 0325-0329
						+										*030102
	+					+									+	*0304
						+										*0308, 29010101-290201, 290203, 2904, 2906-2916, 3103, 3104, 3106, 3204
						+										*0310, 1104
						+										*0317
						+										*0324
																*110101-110107, 1103, 1105-1107, 1111-1113, 1115, 1120, 1121N, 1126, 1128-1130
																*110201-110202, 1114, 1116-1119
					+											*1108
																*1109
																*1110
																*1124
	+					+										*1127
	+															*2417, 2441
																*2418
+													+			*250101-2503, 2505, 2506, 260101-260104, 2602, 2603, 2605-260702, 2609, 2610, 2612, 2613, 2615-2617, 2619-2621, 2623-2626, 2629-2634, 4301, 6603, 6605
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.

Length of spec.	195	435	195	180	140	225	135	160	160	165	160	405	215	195	145	150
PCR product				220	235		155				200				205	215
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2504, 260105, 2608, 2627																
*2604																
*2622																
*2905										+						
*3401, 3405, 6601, 6602, 6604	+															
*3402-3404, 3407, 3408	+	+														
*3601-3603													f			
*3604													f			
*6901	+															
*8001									+							
HLA-A allele ⁴																
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

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*68 subtyping. . In addition, tubes number 5, 10, 11, 22 and 29 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 1st, 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.

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 sequences of the A*68020103 and A*6839 alleles are not yet retrievable.

165	195	325	90	165	165	185	375	240	185	190	200	255	145	205	225	Length of spec.
185	225			215									245			PCR product
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.
													+			2504, 260105, 2608, 2627
+										+			+			*2604
+							+						+			*2622
						+										*2905
+													+			*3401, 3405, 6601, 6602, 6604
						+										*3402-3404, 3407, 3408
					W	+										*3601-3603
					W											*3604
							+									*6901
	+															*8001
																HLA-A allele ⁴
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.

⁵Primer mix 4: Specific PCR fragment of 180 bp in the A*6830 allele. Specific PCR fragment of 220 bp in the A*680301-680302, A*6805 and A*6820 alleles.

Primer mix 5: Specific PCR fragment of 140 bp in the A*6825 allele. Specific PCR fragment of 235 bp in the A*6804 allele.

Primer mix 7: Specific PCR fragment of 135 bp in the A*6806 and A*0252 alleles. Specific PCR fragment of 155 bp in the A*6832 allele.

Primer mix 11: Specific PCR fragment of 160 bp in the A*6817 allele. Specific PCR fragment of 200 bp in the A*6810 and A*6814 alleles.

Primer mix 15: Specific PCR fragment of 145 bp in the A*6819 and A*0317, A*2418 alleles. Specific PCR fragment of 205 bp in the A*6814 allele.

Primer mix 16: Specific PCR fragment of 150 bp in the A*6816 allele. Specific PCR fragment of 215 bp in the A*6815 and A*6831 alleles.

Primer mix 17: Specific PCR fragment of 165 bp in the A*68020101-68020102, A*6815, A*6818N, A*6827, A*6828, A*6831, A*6834 and A*0202, A*020301-020302, A*0205, A*0208, A*0222, A*0247, A*0249, A*0250, A*0263, A*9202, A*9204, A*9215, A*2302, A*2406, A*250101-2503, A*2505, A*2506, A*260101-260104, A*2602-260702, A*2609-2626, A*2628-2634, A*3210, A*3401, A*3405, A*4301, A*6601 to A*6605 alleles. Specific PCR fragment of 185 bp in the A*6820 allele.

Primer mix 18: Specific PCR fragment of 195 bp in the A*6826 and A*01010101-0104N, A*0106-0125, A*0318, A*1127A*2417, A*2441, A*2903 and A*8001 alleles. Specific PCR fragment of 225 bp in the A*6821 and A*0304 alleles.

Primer mix 21: Specific PCR fragment of 165 bp in the A*6828 and A*0249 alleles. Specific PCR fragment of 215 bp in the A*6824 allele.

Primer mix 30: Specific PCR fragment of 145 bp in the A*250101-2506, A*260101-2610, A*2612, A*2613, A*2615-2617, A*2619-2627, A*2629-2634, A*3401, A*3406, A*4301, A*6601-6606 alleles. Specific PCR fragment of 245 bp in the A*6837 allele.

'w', may be weakly amplified.

'f', may be faintly amplified.

'?', nucleotide sequence information not available for the primer matching sequence.

CELL LINE VALIDATION SHEET																			
HLA-A*68 SSP subtyping kit																			
			Prod. No.:	Tube															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	cell line	A*		200732601	200732602	200732603	200732604	200732605	200732606	200732607	200732608	200732609	200732610	200732611	200732612	200732613	200732614	200732615	200732616
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	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-

CELL LINE VALIDATION SHEET																					
HLA-A*68 SSP subtyping kit																					
				Prod. No.:	Tube																
					17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
	cell line	A*			200732617	200732618	200732619	200732620	200732621	200732622	200732623	200732624	200732625	200732626	200732627	200732628	200732629	200732630	200732631	200732632	
1	9001 SA	*2402			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707	*0201			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324	*0101			-	+	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373	*3001			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*0101			-	+	-	-	-	W	-	-	-	-	-	-	-	-	-	-	-
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			W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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		-	+	-	-	-	W	+	-	-	-	-	-	-	-	-	-	-
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*68 SSP

Product number: 101.418-12/04 – licensed for PCR
101.418-12u/04u – not licensed for PCR
Lot number: X65
Expiry date: 2009-May-01
Number of tests: 12 tests – Product No. 101.418-12
4 tests – Product No. 101.418-04
Number of tubes per test: 32

Tube specifications:

Tube No.	Production No.	Tube No.	Production No.	Tube No.	Production No.
1	2007-326-01	13	2007-326-13	25	2007-326-25
2	2007-326-02	14	2007-326-14	26	2007-326-26
3	2007-326-03	15	2007-326-15	27	2007-326-27
4	2007-326-04	16	2007-326-16	28	2007-326-28
5	2007-326-05	17	2007-326-17	29	2007-326-29
6	2007-326-06	18	2007-326-18	30	2007-32630
7	2007-326-07	19	2007-326-19	31	2007-326-31
8	2007-326-08	20	2007-326-20	32	2007-326-32
9	2007-326-09	21	2007-326-21		
10	2007-326-10	22	2007-326-22		
11	2007-326-11	23	2007-326-23		
12	2007-326-12	24	2007-326-24		

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

No DNAs carrying the alleles to be amplified by primer solutions 4, 5, 6, 7, 8, 11 to 16, 19 to 21, 25 to 29 and 31 and 32 were available. The specificities of the primers in primer solutions 5, 7, 13, 20, 25, 26 and 29 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. The specificities of the 3'-primers in primer solutions 4, 6, 8, 12, 19, 28 and 31 were tested by separately adding one additional 5'-primer, it was not possible test the 5'-primers. The specificity of the 5'-primer in primer solutions 14, 27 and 32 were tested by separately adding one additional 3'-primer, it was not possible test the 3'-primers. In primer solution 11 one of the 5'-primers was not possible to test. In primer solutions 15 and 16 one of the 3'-primers was not possible to test. In primer solution 21 it was not possible to test either the 5'-primer or the 3'-primer.

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

Date of approval: 2007-July-16

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: Olerup SSP™ HLA-A*68
Product number: 101.418-12/04, 101.418-12u/04u
Lot number: X65

Intended use: HLA-A*68 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-July-16

Olle Olerup
Managing Director

HLA-A*68
101.418-12/04 – licensed for PCR
101.418-12u/04u – not licensed for PCR
Lot No.: **X65**

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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.

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*68
101.418-12/04 – licensed for PCR
101.418-12u/04u – not licensed for PCR
Lot No.: **X65**

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