

Olerup SSP™ HLA-Cw*03

Product number: 101.611-12 – licensed for PCR
101.611-12u – not licensed for PCR
Lot number: Y28
Expiry date: 2009-September-01
Number of tests: 12
Number of tubes per test: 31
Storage - pre-aliquoted primers: dark at -20°C
- PCR Master Mix: -20°C

This Product Description is only valid for Lot No. Y28.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP™ HLA-Cw*03 LOT

The HLA-Cw*03 specificity and interpretation tables have been updated for the HLA-Cw alleles described since the previous *Olerup SSP™* HLA-Cw*03 lot was made (**Lot No. V88**).

Eight tubes have been added to the HLA-Cw*03 kit,
wells **24 to 31**.

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

Tube	5'-primer	3'-primer	rationale
4	-	Added	Modified primer added for Cw*030407.
6	Modified	-	Increased yield of specific PCR product.
11	Exchanged	-	Primer exchanged to avoid co-amplification of Cw*15 alleles.
15	-	Modified	Increased yield of specific PCR product.
19	-	Added	Primer added for the Cw*0333 allele.
24	New	New	Primer pairs for the Cw*0332 and 0338 alleles.
25	New	New	Primer pairs for the Cw*0334, 0335 and 0336 alleles.
26	New	New	Primer pairs for the Cw*0334, 0335 and 0336 alleles.
27	New	New	Primer pair for the Cw*0337 allele.
28	New	New	Primer pair for the Cw*0341 allele.
29	New	New	Primer pair for the Cw*0339 allele.
30	New	New	Primer pair for the Cw*0346 allele.
31	New	New	Primer pair for the Cw*0344 allele.

PRODUCT DESCRIPTION

HLA-Cw*03 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-Cw*0302 to Cw*0346 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.

31 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 31 PCR reactions in a 32 well cut PCR plate. Well 32 is empty.

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	empty

The 32 well cut PCR plate is marked with 'Cw*03 Y28'.

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-Cw*03 SSP subtypings will be influenced by the one Cw*01, the Cw*02, the Cw*04, the Cw*06, several Cw*07, two Cw*12, the Cw*14, the Cw*15, the Cw*17 and Cw*18 alleles when present on the other haplotype. In addition, the B*1596 allele is amplified by primer mixes 5, 6 and 26.

UNIQUELY IDENTIFIED ALLELES

All the HLA-C*03 alleles, i.e. **Cw*0302 to Cw*0346**, recognized by the HLA Nomenclature Committee in October 2007¹ will give rise to unique amplification patterns by the primers in the HLA-Cw*03 subtyping kit.

The HLA-Cw*03 subtyping kit cannot distinguish the Cw*030201 to Cw*030203 alleles, the Cw*030301 to Cw*030305 alleles, the Cw*030401 to Cw*030407 alleles or the Cw*033801 and Cw*033802 alleles.

¹**Nomenclature for factors of the HLA system, 1998.** *Tissue Antigens* 1999; **53**: 407-446.
HLA-Cw alleles listed on the IMGT/HLA web page 2007-October-05, release 2.19.9, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 45 HLA-Cw*03 alleles will give rise to 46 different amplification patterns that can be combined in 1 081 homozygous and heterozygous combinations. 313 of these genotypes do not give rise to unique amplification patterns. The different sizes of the specific PCR products generated by the primers in vials 6, 7, 13, 19, 25 and 26 have not been considered in these calculations.

```
+++++--+ - - - - + - + - - - - - - - - + - - - - - 0313,0340 = 0335,0343
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0305,0343 = 0317,0343
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0321 = 0306,0343 = 0321,0340
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0322Q = 0303,0342 = 0309,0343 =
0322Q,0340 = 0322Q,0342 = 0342,0343
+++++--+ + + - - + - + + - - - - - + - - - - - 0303,0315 = 0315,0343
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0331 = 0308,0343 = 0331,0340
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,031101 = 031101,0340
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0312 = 0312,0340 = 0319,0343
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0320N = 0320N,0340
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0303,0333 = 0324,0343 = 0333,0343
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0330 = 0330,0340
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0303,0336 = 0334,0343 = 0336,0343
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,031102 = 031102,0340 = 0337,0343
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0316,0343 = 0341,0343
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0303 = 0302,0343 = 0303,0340 =
0304,0343 = 0314,0343 = 0340,0343
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0313 = 0313,0336
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0305,0342 = 0317,0342
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0305,0340 = 0314,0317 = 0317,0340
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0306 = 0306,0340
+++++--+ + + - - + - + + - - - - - + - - - - - 0308,0315 = 0315,0329
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0329 = 0329,0340
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0308 = 0308,0340
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0339 = 0339,0340
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0318 = 0318,0340
+++++--+ + + - - + - + + - - - - - + - - - - - 0309,0315 = 0315,0342
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0309,0333 = 0324,0342 = 0333,0342
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0309,0336 = 0334,0342 = 0336,0342
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0316,0342 = 0341,0342
+++++--+ - - - - + - + - - - - - - - - - - - - - - 0302,0309 = 0302,0342 = 0304,0342 =
0309,0340 = 0309,0342 = 0314,0342 =
0340,0342 = 0342,0342
+++++--+ + + - - + - + + - - - - - + - - - - - 0315,0332 = 0315,0338 = 0315,0345
+++++--+ + + - - + - + + - - - - - + - - - - - 0304,0315 = 0307,0315 = 0310,0315 =
0314,0315 = 0315,0340
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+++-----	++-+---+	-----	+-----	0302,0345 = 0340,0345
++-+-----	++-+---+	-----	-----	0302,0307 = 0307,0340
+++-----	-+---+---	-----	-----	0302,0310 = 0310,0340
+++-----	-----+	-----	-----	0302,0319 = 0319,0340
+++-----	-----+	-+-----	-----	0302,0323 = 0323,0340
+++-----	-----+	-+-----	-+-----	0324,0336 = 0333,0334
+++-----	-----+	-+-----	-----	0302,0324 = 0304,0333 = 0314,0333 =
				0324,0333 = 0324,0340 = 0333,0340
++-+-----	-----+	-----+	-----	0302,0326 = 0326,0340
++-+-----	-----+	-----+	+-----	0302,0338 = 0338,0340
++-+-----	-----+	-----+	-----	0302,0328 = 0328,0340
++-+-----	-----+	-----+	+-----	0302,0332 = 0332,0340
+++-----	-----+	-----	-+-----	0302,0334 = 0304,0336 = 0314,0336 =
				0334,0336 = 0334,0340 = 0336,0340
+++-----	-----+	-----	-+-----	0302,0337 = 0337,0340
+++-----	-----+	-----	-+-----	0314,0316 = 0316,0340 = 0340,0341
++-+-----	-----+	-----	-----+	0302,0346 = 0340,0346
++-+-----	-----+	-----	-----+	0302,0344 = 0340,0344
+++-----	-----+	-----	-----	0302,0304 = 0302,0314 = 0302,0340 =
				0304,0340 = 0314,0340 = 0340,0340
+++-----	-----+	-----	-----	0304,0314 = 0314,0314
++-+-----	++-+---+	-----+	-----	0305,0315 = 0315,0317 = 0315,0327
++-+-----	-----+	-+-----	-----	0305,0333 = 0317,0333
++-+-----	-----+	-----	-+-----	0302,0335 = 0305,0336 = 0317,0336 =
				0335,0336
++-+-----	-----+	-----	-----	0302,0305 = 0302,0317
+++-----	++-+---+	-----+	-+-----	0315,0316 = 0315,0341
+++-----	++-+---+	-----+	-----	0302,0315 = 0315,0315
+++-----	-----+	-+-----	-----+	0316,0333 = 0333,0341
+++-----	-----+	-+-----	-----	0302,0333 = 0333,0333
+++-----	-----+	-----	-+-----	0316,0336 = 0336,0341
+++-----	-----+	-----	-+-----	0302,0336 = 0336,0336
+++-----	-----+	-----	-+-----	0302,0316 = 0302,0341
+++++-----	-----+	-----	-+-----	0306,0313 = 0321,0335
+++++-----	-----+	-----	-+-----	0309,0313 = 0322Q,0335
+++++-----	-----+	-----	-+-----	0308,0313 = 0331,0335
+++++-----	-----+	-----	-+-----	0312,0335 = 0313,0319
+++++-----	-----+	-----	-+-----	031102,0335 = 0313,0337
+++++-----	-----+	-----	-+-----	0303,0335 = 0304,0313 = 0313,0334
+++++-----	-----+	-----	-----	0306,0322Q = 0309,0321
+++++-----	-+-----	-----	-----	0306,0331 = 0308,0321
+++++-----	-----+	-----	-----	0306,0312 = 0319,0321
+++++-----	-----+	-----	-+-----	0306,031102 = 0321,0337
+++++-----	-----+	-----	-----	0303,0306 = 0304,0321 = 0306,0321
+++++-----	-+-----	-----	-----	0308,0322Q = 0309,0331
+++++-----	-----+	-----	-----	0309,0312 = 0319,0322Q
+++++-----	-----+	-----	-+-----	0309,031102 = 0322Q,0337
+++++-----	-----+	-----	-----	0303,0309 = 0304,0322Q = 0309,0322Q
+++++-----	-+-----	-----	-----	0303,0329 = 0310,0331 = 0329,0331
+++++-----	-+-----	-----	-----	0308,0312 = 0319,0331
+++++-----	-+-----	-----	-+-----	0308,031102 = 0331,0337
+++++-----	-+-----	-----	-----	0303,0308 = 0304,0331 = 0308,0331
+++++-----	-----+	-----	-+-----	0304,031101 = 031101,0337
+++++-----	-----+	-----	-+-----	031102,0319 = 0312,0337
+++++-----	-----+	-----	-----	0303,0319 = 0304,0312 = 0312,0319
+++++-----	-----+	-----	-+-----	0303,0337 = 0304,031102 = 031102,0337
+++++-----	-----+	-----	-+-----	0305,0313 = 0313,0335
+++++-----	-----+	-----+	+-----	0305,0338 = 0327,0332 = 0327,0338
+++++-----	-----+	-----	-+-----	0304,0335 = 0305,0334 = 0334,0335

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+---+---+---	-----+--	-----	-----	0304,0306 = 0306,0306
+---+---+---	+++-----	-----	+-----	0308,0345 = 0329,0345
+---+---+---	+++-----	-----	-----	0307,0308 = 0307,0329
+---+---+---	-++-----	-----	-----	0304,0329 = 0308,0310 = 0308,0329 = 0310,0329 = 0329,0329
+---+---+---	--+-----	-----	-----	0304,0308 = 0308,0308
+---+---+---	-----+--	-----	-----	0304,0309 = 0309,0309
+---+---+---	+++-----	-----+--	+-----	0307,0338 = 0338,0345
+---+---+---	+++-----	-----	+-----	0304,0345 = 0307,0332 = 0307,0345 = 0310,0345 = 0332,0345 = 0345,0345
+---+---+---	+++-----	-----	-----	0304,0307 = 0307,0307 = 0307,0310
+---+---+---	-+-----	-----	-----	0304,0310 = 0310,0310
+---+---+---	-----++-	-----	-----	0304,0319 = 0319,0319
+---+---+---	-----+--	-+-----	-----	0304,0323 = 0323,0323
+---+---+---	-----+--	--+-----	-----	0304,0324 = 0324,0324
+---+---+---	-----+--	-----+--	-----	0304,0326 = 0326,0326
+---+---+---	-----+--	-----+--	+-----	0304,0338 = 0332,0338 = 0338,0338
+---+---+---	-----+--	-----+--	-----	0304,0328 = 0328,0328
+---+---+---	-----+--	-----	+-----	0304,0332 = 0332,0332
+---+---+---	-----+--	-----	-+-----	0304,0334 = 0334,0334
+---+---+---	-----+--	-----	--+-----	0304,0337 = 0337,0337
+---+---+---	-----+--	-----	-----+--	0304,0346 = 0346,0346
+---+---+---	-----+--	-----	-----+--	0304,0344 = 0344,0344
+---+---+---	-----+--	-----	-----+--	0305,0316 = 0316,0317 = 0317,0341
+---+---+---	-----+--	-----	-----	0305,0317 = 0317,0317
+---+---+---	-----+--	-----+--	-----	0305,0325 = 0325,0325
+---+---+---	-----+--	-----+--	-----	0305,0327 = 0327,0327
+---+---+---	-----+--	-----	-+-----	0305,0335 = 0335,0335
+---+---+---	-----+--	-----	-----+--	0316,0316 = 0316,0341
-+++-----	-----+--	-----	-----	0303,0343 = 0343,0343
--+++-----	-----+--	-----	-----	0303,0321 = 0321,0321
--+++-----	-----+--	-----	-----	0303,0322Q = 0322Q,0322Q
--+++-----	--+-----	-----	-----	0303,0331 = 0331,0331
--+++-----	-----+--	-----	--+-----	031101,0339 = 031102,0339
--+++-----	-----+--	-----	--+-----	0303,031101 = 031101,031101 = 031101,031102 = 031101,0318 = 031102,0318
--+++-----	-----+--	-----	-----	0303,0312 = 0312,0312
--+++-----	-----+--	+-----	-----	0303,0320N = 0320N,0320N
--+++-----	-----+--	-----+--	-----	0303,0330 = 0330,0330
--+++-----	-----+--	-----	--+-----	0303,031102 = 031102,031102
--+++-----	-----+--	-----	-----+--	0318,0339 = 0339,0339

LICENSES

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101.611-12 and 101.611-12u

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-Cw*03 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-Cw*03 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 31 wells of a HLA-Cw*03 subtyping. **Well No. 1 of the 32 well PCR plate is marked with '1'**. Close the 32 well PCR plate with the provided lids.

101.611-12u – not licensed for PCR

For one HLA-Cw*03 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 31 wells of an HLA-Cw*03 subtyping. **Well No. 1 of the 32 well PCR plate is marked with '1'**. Close the 32 well PCR plate with the provided lids.

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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-Cw*03 SSP subtyping

Specificities and sizes of the PCR products of the 31 primer mixes used for HLA-Cw*03 SSP subtyping

Primer Mix	Approx. size of spec. PCR product ¹	Size of control band ²	Amplified HLA-Cw*03 alleles ³	Other amplified HLA Class I alleles ⁴
1⁵	280 bp	800 bp	030201-030203, 030401-0310, 0314-0317, 0319, 0323-0329, 0332-033802, 0340-0342, 0344-0346	020201-020203, 020205-0203, 0204 ^{weakly} , 0205-0213, 0214 ^{weakly} , 0215-0220, 0403, 0406, 0416, 0603, 150201-1511, 1513, 1515-1521
2	210 bp	1070 bp	030201-030203, 0314, 0315, 0333, 0336, 0340, 0342, 0343	
3	280 bp	800 bp	030301-030305, 031101-0313, 0320N-0322Q, 0330, 0331, 0343	1512
4	275 bp	1070 bp	030301-030407, 0306-0312, 0314, 0318-0324, 0326, 0328-0332, 0334, 0337-0340, 0342-0346	
5	275 bp	1070 bp	0305, 0313, 0317, 0325, 0327, 0335	B*1596
6^{6,9}	90, 215 bp	800 bp	0306, 0321	B*1596
7^{6,10}	110, 185 bp	1070 bp	030301-030305, 0308, 031101-0313, 0318, 0320N-0322Q, 0329-0331, 0339, 0343	070101-070109, 0706, 0707, 0709, 0716, 0718-0720, 0722, 0724, 0726, 0728, 0730, 0735, 0736, 0740, 0744, 0752, 0753
8	250 bp	1070 bp	0309, 0322Q, 0342	
9	240 bp	1070 bp	0307, 0315, 0345	020201-020203,

				020205-0203, 0204 ^{weakly} , 0205- 0211, 0213, 0214 ^{weakly} , 0215- 0220, 0403, 0406, 0416, 0603, 150201-1506, 1508-1513, 1515- 1520
10⁷	250 bp	800 bp	0307, 0310, 0315, 0329, 0345	020201 ^{weakly} , 020202-020203, 020205-0203, 0204 ^{weakly} , 0205- 0213, 0214 ^{weakly} , 0215-0220, 0403, 0406, 0416, 0603, 150201-1506, 1508-1513, 1515- 1521
11^{6,7}	415 bp	1070 bp	0308, 0329, 0331	1208
12	260 bp	1070 bp	031101, 0318, 0339	1512
13^{6,11}	80, 140 bp	1070 bp	030201-030203, 0315-0317, 0333, 0336, 0340, 0342, 0343	140201, 140203- 1409
14⁶	210 bp	1070 bp	0312, 0319	
15⁶	135 bp	1070 bp	030201-0317, 0319-033802, 0340-0346	0603 ^{weakly}
16	150 bp	1070 bp	0307, 0310, 0315, 0329, 0345	0114, 06020101- 0604, 0606-0610, 0612-0616N, 0707, 0709, 120401, 1701- 1704, 1801-1803
17^{6,7}	90 bp	1070 bp	0320N	
18	225 bp	1070 bp	0323	
19^{6,12}	105, 260 bp	800 bp	0324, 0333	
20	165 bp	1070 bp	0325	
21	210 bp	1070 bp	0326	
22⁵	195 bp	800 bp	0315, 0327, 033801-033802	0212, 0403, 0406, 0416, 0720, 1503, 1516
23⁶	90 bp	1070 bp	0328	
24	200 bp	1070 bp	0330	
25¹³	295, 345	1070 bp	0332, 033801-	0614, 0710, 0743,

26 ^{6,14}	bp		033802, 0345	1503, 1516
	70, 105, 265 bp	1070 bp	0313, 0334-0336	B*1596
27 ⁶	80 bp	1070 bp	031101-031102, 0337	0603
28 ⁸	170 bp	1070 bp	0316, 0341	04010101-040104, 0403-0420, 0423- 0431, 140201, 140203-1409, 1801-1803
29	285 bp	1070 bp	0339	
30	165 bp	1070 bp	0346	
31	210 bp	800 bp	0344	

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-Cw*03 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-Cw*03 subtyping.

In addition, tubes number 3, 6, 11, 19, 22 and 31 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.

⁴Due to the sharing of sequence motifs between HLA-Cw alleles the primer pairs in tubes 1, 3, 7, 9 to 13, 15, 16 and 22 will amplify some non-HLA-Cw*03 alleles. In addition, the B*1596 allele will be amplified by primer mixes 5, 6 and 26

⁵The primer pairs in tubes 1 and 22 may give rise to less specific PCR product than the other HLA-Cw*03 primer pairs.

⁶Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁷Primer mixes 10, 11 and 17 may give rise to primer oligomer formation, most pronounced for primer mix 11.

⁸Primer mix 28 may give rise to nonspecific amplifications.

⁹Primer mix 6: Specific PCR fragment of 90 bp in the Cw*0321 and B*1596 alleles. Specific PCR fragment of 215 bp in the Cw*0306 alleles.

¹⁰Primer mix 7: Specific PCR fragment of 110 bp in the Cw*0308, 0329 and 0331 and the Cw*070101-070109, 0706, 0707, 0709, 0716, 0718-0720, 0722, 0724, 0726, 0728, 0730, 0735, 0736, 0740, 0744, 0752 and 0753 alleles. Specific PCR fragment of 185 bp in the Cw*030301-030305, 031101-0313, 0318, 0320N to 0322Q, 0329 to 0331, 0339 and 0343 alleles.

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¹¹Primer mix 13: Specific PCR fragment of 80 bp in the Cw*030201-030203, 0315, 0316, 0333, 0336, 0340, 0342 and 0343 and the Cw*140201, 140203 to 1409 alleles. Specific PCR fragment of 140 bp in the Cw*0317 allele.

¹²Primer mix 19: Specific PCR fragment of 105 bp in the Cw*0324 allele. Specific PCR fragment of 260 bp in the Cw*0333 allele.

¹³Primer mix 25: Specific PCR fragment of 295 bp in the Cw*0332 and 0345 and the Cw*0614, 0710 and 0743 alleles. Specific PCR fragment of 345 bp in the Cw*033801-033802 and the Cw*1503 and 1516 alleles.

¹⁴Primer mix 26: Specific PCR fragment of 70 bp in the Cw*0334 allele. Specific PCR fragment of 105 bp in the Cw*0336 allele. Specific PCR fragment of 265 bp in the Cw*0313 and 0335 and the B*1596 alleles.

INTERPRETATION TABLE																
HLA-Cw*03 SSP subtyping																
Amplification patterns of the Cw*0302 to Cw*0346 alleles																
	Tube⁵															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length of spec.	280	210	280	275	275	90	110	250	240	250	415	260	80	210	135	150
PCR product						215	185						140			
Length of int.	800	1070	800	1070	1070	800	1070	1070	1070	800	1070	1070	1070	1070	1070	1070
pos. control¹																
5'-primer(s)²	106	419	105	355	355	413	201	374	105	105	28	126	361	420	105	201
	5'-CTg ³	5'-gTC ³	5'-gCT ³	5'-TCA ³	5'-CCC ³	5'-TgT ³	5'-CCg ³	5'-CTA ³	5'-gCT ³	5'-gCT ³	5'-TCA ³	5'-ggA ³	5'-AgT ³	5'-TTA ³	5'-gCT ³	5'-CCg ³
						539		379					419			
						5'-gTg ³		5'-ACC ³					5'-gTC ³			
3'-primer(s)³	343	589	343	589	589	589	270	589	302	312	270	343	459	589	201	312
	5'-ACC ³	5'-CTT ³	5'-ACT ³	5'-CTT ³	5'-CTT ³	5'-CTT ³	5'-TAG ³	5'-CTT ³	5'-ggT ³	5'-AgT ³	5'-TAG ³	5'-ACT ³	5'-AgA ³	5'-CTT ³	5'-CTC ³	5'-AGT ³
							343									
							5'-ACT ³									
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
HLA-Cw* allele⁴																
*030201-030203	+	+											+		+	
*030301-030305			+	+			+								+	
*030401-030407	+			+											+	
*0305	+				+										+	
*0306	+			+		+									+	
*0307	+			+					+	+					+	+
*0308	+			+			+				+				+	
*0309	+			+				+							+	
*0310	+			+						+					+	+
*031101			+	+			+					+			+	
*031102			+	+			+								+	
*0312			+	+			+							+	+	
*0313			+		+		+								+	
*0314	+	+		+											+	
*0315	+	+							+	+			+		+	+
*0316	+												+		+	
*0317	+				+								+		+	
*0318				+			+					+				
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

INTERPRETATION TABLE															
HLA-Cw*03 SSP subtyping															
Amplification patterns of the Cw*0302 to Cw*0346 alleles															
Tube ⁵															
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
90	225	105	165	210	195	90	200	295	70	80	170	285	165	210	Length of spec.
		260						345	105						PCR product
									265						
1070	1070	800	1070	1070	800	1070	1070	1070	1070	1070	1070	1070	1070	800	Length of int.
															pos. control ¹
19	406	105	467	420	134	539	355	289	363	105	459	97	105	421	5'-primer(s) ²
5'-CCT ³	5'-gCA ³	5'-gCT ³	5'-CTT ³	5'-TTC ³	5'-CCA ³	5'-gCg ³	5'-TCA ³	5'-Agg ³	5'-Agg ³	5'-gCT ³	5'-gAT ³	5'-TCg ³	5'-gCT ³	5'-ACC ³	
								341	527						
								5'-ggA ³	5'-TgT ³						
								560							
								5'-CgA ³							
70	589	167	589	589	289	589	514	353	589	142	589	343	231	589	3'-primer(s) ³
5'-ggC ³	5'-CTT ³	5'-ACC ³	5'-CTT ³	5'-CTT ³	5'-AgC ³	5'-CTT ³	5'-CTT ³	5'-TgA ³	5'-CTT ³	5'-TgA ³	5'-CTC ³	5'-ACT ³	5'-CTC ³	5'-CTT ³	
		323													
		5'-AgC ³													
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Tube No.
															HLA-Cw* allele ⁴
															*030201-030203
															*030301-030305
															*030401-030407
															*0305
															*0306
															*0307
															*0308
															*0309
															*0310
										+					*031101
										+					*031102
															*0312
									+						*0313
															*0314
					+										*0315
											+				*0316
															*0317
															*0318
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Tube No.

Length of spec.	280	210	280	275	275	90	110	250	240	250	415	260	80	210	135	150
PCR product						215	185						140			
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*0319	+			+										+	+	
*0320N			+	+			+								+	
*0321			+	+		+	+								+	
*0322Q			+	+			+	+							+	
*0323	+			+											+	
*0324	+			+											+	
*0325	+				+										+	
*0326	+			+											+	
*0327	+				+										+	
*0328	+			+											+	
*0329	+			+			+			+	+				+	+
*0330			+	+			+								+	
*0331			+	+			+				+				+	
*0332	+			+											+	
*0333	+	+											+		+	
*0334	+			+											+	
*0335	+				+										+	
*0336	+	+											+		+	
*0337	+			+											+	
*033801-033802	+			+											+	
*0339				+			+					+				
*0340	+	+		+									+		+	
*0341	+														+	
*0342	+	+		+				+					+		+	
*0343		+	+	+			+						+		+	
*0344	+			+											+	
*0345	+			+					+	+					+	+
*0346	+			+											+	
*0114, 06020101- 060203, 0604, 0606- 0610, 0612, 0613, 0615, 0616N, 120401, 1701-1704																+
*020201	+								+	W						
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

90	225	105	165	210	195	90	200	295	70	80	170	285	165	210	Length of spec. PCR product
		260						345	105						
									265						
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Tube No.
															*0319
+															*0320N
															*0321
															*0322Q
	+														*0323
		+													*0324
			+												*0325
				+											*0326
					+										*0327
						+									*0328
							+								*0329
								+							*0330
									+						*0331
										+					*0332
		+													*0333
										+					*0334
										+					*0335
										+					*0336
											+				*0337
					+			+							*033801-033802
												+			*0339
															*0340
											+				*0341
															*0342
															*0343
														+	*0344
								+							*0345
													+		*0346
															*0114, 06020101- 060203, 0604, 0606- 0610, 0612, 0613, 0615, 0616N, 120401, 1701-1704
															*020201
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Tube No.

Length of spec.	280	210	280	275	275	90	110	250	240	250	415	260	80	210	135	150
PCR product						215	185						140			
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*020202-020203, 020205-0203, 0205- 0211, 0213, 0215- 0220, 150201- 150203, 1504-1506, 1508-1511, 1513, 1515, 1517-1520	+								+	+						
*0204, 0214	W								W	W						
*0212	+									+						
*04010101-040104, 040401, 0405, 0407- 041502, 0417-0420, 0423-0431																
*0403, 0406, 0416	+								+	+						
*0603	+								+	+					W	+
*0614																+
*070101-070109, 0706, 0716, 0718, 0719, 0722, 0724, 0726, 0728, 0730, 0735, 0736, 0740, 0744, 0752, 0753							+									
*0707, 0709							+									+
*0710, 0743																
*0720							+									
*1208											+					
*140201, 140203- 1409													+			
*1503, 1516	+								+	+						
*1507	+															
*1512			+						+	+		+				
*1521	+									+						
*1801-1803																+
HLA-Cw* allele ⁴																
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
B*1596					+	+										
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

HLA-Cw*03
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90	225	105	165	210	195	90	200	295	70	80	170	285	165	210	Length of spec. PCR product
		260						345	105						
									265						
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Tube No.
															*020202-020203, 020205-0203, 0205- 0211, 0213, 0215- 0220, 150201- 150203, 1504-1506, 1508-1511, 1513, 1515, 1517-1520
															*0204, 0214
					+										*0212
											+				*04010101-040104, 040401, 0405, 0407- 041502, 0417-0420, 0423-0431
					+						+				*0403, 0406, 0416
										+					*0603
															*0614
															*070101-070109, 0706, 0716, 0718, 0719, 0722, 0724, 0726, 0728, 0730, 0735, 0736, 0740, 0744, 0752, 0753
															*0707, 0709
									+						*0710, 0743
					+										*0720
															*1208
											+				*140201, 140203- 1409
					+				+						*1503, 1516
															*1507
															*1512
															*1521
											+				*1801-1803
															HLA-Cw* allele ⁴
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Tube No.
									+						B*1596
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	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-Cw*03 subtyping.

In addition, tubes number 3, 6, 10, 19, 22 and 31 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 exons, 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 1st, 2nd or 3rd exons, 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 HLA-Cw*0301 allele has been shown to be identical to Cw*0304.

⁵Primer mix 6: Specific PCR fragment of 90 bp in the Cw*0321 and B*1596 alleles. Specific PCR fragment of 215 bp in the Cw*0306 alleles.

Primer mix 7: Specific PCR fragment of 110 bp in the Cw*0308, 0329 and 0331 and the Cw*070101-070109, 0706, 0707, 0709, 0716, 0718-0720, 0722, 0724, 0726, 0728, 0730, 0735, 0736, 0740, 0744, 0752 and 0753 alleles. Specific PCR fragment of 185 bp in the Cw*030301-030305, 031101-0313, 0318, 0320N to 0322Q, 0329 to 0331, 0339 and 0343 alleles.

Primer mix 13: Specific PCR fragment of 80 bp in the Cw*030201-030203, 0315, 0316, 0333, 0336, 0340, 0342 and 0343 and the Cw*140201, 140203 to 1409 alleles. Specific PCR fragment of 140 bp in the Cw*0317 allele.

Primer mix 19: Specific PCR fragment of 105 bp in the Cw*0324 allele. Specific PCR fragment of 260 bp in the Cw*0333 allele.

Primer mix 25: Specific PCR fragment of 295 bp in the Cw*0332 and 0345 and the Cw*0614, 0710 and 0743 alleles. Specific PCR fragment of 345 bp in the Cw*033801-033802 and the Cw*1503 and 1516 alleles.

Primer mix 26: Specific PCR fragment of 70 bp in the Cw*0334 allele. Specific PCR fragment of 105 bp in the Cw*0336 allele. Specific PCR fragment of 265 bp in the Cw*0313 and 0335 and the B*1596 alleles.

'w', might be weakly amplified.

CELL LINE VALIDATION SHEET																			
HLA-Cw*03 SSP subtyping kit																			
				Tube															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.:															
				200737001	200737002	200737003	200737004	200737005	200737006	200737007	200737008	200737009	200737010	200737011	200737012	200737013	200737014	200737015	200737016
	cell line		Cw*																
1	9001	SA	*0702	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*0701	*1505	+	-	-	-	-	-	+	-	+	+	+	-	-	-	-
3	9011	E4181324	*1202		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*0304	*0401	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-
5	9009	KAS011	*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
6	9353	SM	*0304	*0702	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-
7	9020	QBL	*0501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9007	DEM	*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
9	9026	YAR	*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*0102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
13	9067	BTB	*0102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*0102	*0304	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-
15	9075	DKB	*0304		+	-	-	+	-	-	-	-	-	-	-	-	-	+	-
16	9037	SWEIG007	*0202		+	-	-	-	-	-	-	+	+	-	-	-	-	-	-
17	9008	WILJON	*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*0102	*0705	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*0701		-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
20	9059	SLE005	*0304		+	-	-	+	-	-	-	-	-	-	-	-	-	+	-
21	9064	AMALA	*0303		-	-	+	+	-	-	+	-	-	-	-	-	-	+	-
22	9056	KOSE	*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*0102	*1502	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-
24	9035	JBUSH	*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*0802		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*0701		-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
27	9191	CH1007	*0704	*1505	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-
28	9320	BEL5GB	*0501	*1601	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*1701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
31	9019	DUCAF	*0501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*1701	*1703	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
33	9098	MT14B	*0304		+	-	-	+	-	-	-	-	-	-	-	-	-	+	-
34	9104	DHIF	*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*0501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*0303	*0401	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-
37	9065	H-HKB	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*0303		-	-	+	+	-	-	+	-	-	-	-	-	-	+	-
39	9315	CML	*0202	*0701	+	-	-	-	-	-	+	-	+	+	-	-	-	-	-
40	9134	WHONP199	*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
41	9055	H0301	*0802		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*0102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*0102	*0801	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*0602	*1701	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
46	9013	SCHU	*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*0704	*1502	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-
48	9303	TER-ND	*0401	*1601	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CELL LINE VALIDATION SHEET																				
HLA-Cw*03 SSP subtyping kit																				
				Prod. No.:	Tube															
					17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
	cell line		Cw*		200737017	200737018	200737019	200737020	200737021	200737022	200737023	200737024	200737025	200737026	200737027	200737028	200737029	200737030	200737031	
1	9001 SA		*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707		*0701	*1505	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324		*1202		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373		*0304	*0401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011		*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM		*0304	*0702	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL		*0501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9007 DEM		*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR		*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3		*0102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT		*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB		*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9067 BTB		*0102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA		*0102	*0304	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB		*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007		*0202		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9008 WILJON		*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367		*0102	*0705	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16		*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005		*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA		*0303		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE		*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL		*0102	*1502	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH		*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9		*0802		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49		*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007		*0704	*1505	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB		*0501	*1601	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU		*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH		*1701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF		*0501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG		*1701	*1703	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B		*0304		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF		*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO		*0501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17		*0303	*0401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB		*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL		*0303		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML		*0202	*0701	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199		*0602		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301		*0802		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089		*0102		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526		*0102	*0801	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM		*1203		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO		*0602	*1701	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU		*0702		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO		*0704	*1502	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND		*0401	*1601	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP™ HLA-Cw*03 SSP

Product number: 101.611-12 – licensed for PCR
101.611-12u – not licensed for PCR
Lot number: Y28
Expiry date: 2009-September-01
Number of tests: 12
Number of tubes per test: 31

Tube specifications:

Tube No.	Production No.	Tube No.	Production No.	Tube No.	Production No.
1	2007-370-01	13	2007-370-13	25	2007-370-25
2	2007-370-02	14	2007-370-14	26	2007-370-26
3	2007-370-03	15	2007-370-15	27	2007-370-27
4	2007-370-04	16	2007-370-16	28	2007-370-28
5	2007-370-05	17	2007-370-17	29	2007-370-29
6	2007-370-06	18	2007-370-18	30	2007-370-30
7	2007-370-07	19	2007-370-19	31	2007-370-31
8	2007-370-08	20	2007-370-20		
9	2007-370-09	21	2007-370-21		
10	2007-370-10	22	2007-370-22		
11	2007-370-11	23	2007-370-23		
12	2007-370-12	24	2007-370-24		

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 8, 11, 12, 14, 17 to 21, 23 to 26, 29 and 31 were available. The specificities of the primers in primer solutions 8, 11, 14, 21, 23, 25, 26 and 29 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 12, 17, 18, 20 and 31 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutiond 19 and 24 it was only possible to test the 5'-primer, the 3'-primer was not possible to test.

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

Date of approval: 2007-November-22

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: Olerup SSP™ HLA-Cw*03
Product number: 101.611-12, 101.611-12u
Lot number: Y28

Intended use: HLA-Cw*03 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 III, 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.

Saltsjöbaden, Sweden
2007-November-22

Olle Olerup
Managing Director

HLA-Cw*03
101.611-12 – licensed for PCR
101.611-12u – not licensed for PCR
Lot No.: **Y28**

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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-Cw*03
101.611-12 – licensed for PCR
101.611-12u – not licensed for PCR
Lot No.: **Y28**

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