

Olerup SSP™ HLA-Cw*04

Product number: 101.612-12 – licensed for PCR
101.612-12u – not licensed for PCR
Lot number: X77
Expiry date: 2009-April-01
Number of tests: 12
Number of tubes per test: 24
Storage - pre-aliquoted primers: dark at -20°C
- PCR Master Mix: -20°C

This Product Description is only valid for Lot No. X77.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP™ HLA-Cw*04 LOT

The HLA-Cw*04 specificity and interpretation tables have been updated for the HLA-Cw alleles described since the previous Olerup SSP™ HLA-Cw*04 lot was made (Lot No. V19).

Eight tubes have been added to the HLA-Cw*04 kit,
wells **17 to 24**.

The primers of the tubes detailed below has been exchanged or modified.

Tube	5'-primer	3'-primer	rationale
10	Removed	Removed	Cw*0417 primer pair moved to vial 17.
13	Removed	Removed	Cw*0417 primer pair moved to vial 17.
14	-	Modified	Increased yield of specific PCR product.
17	New	New	Cw*0417 primer pair from vials 10 and 13.
18	New	New	New primer pair for the Cw*0419 allele.
19	New	New	New primer pair for the Cw*0420 allele.
20	New	New	New primer pair for the Cw*0421 allele.
21	New	New	New primer pair for the Cw*0423 allele.
22	New	New	New primer pair for the Cw*0424 allele.
23	New	New	New primer pair for the Cw*0425 allele.
24	New	New	New primer pair for the Cw*0426 allele.

PRODUCT DESCRIPTION

HLA-Cw*04 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-Cw*0401 to HLA-Cw*0427 alleles.

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

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

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

24 PCR reactions with a reaction volume of 10 µl are performed per sample.

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

PLATE LAYOUT

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

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

The 24 well cut PCR plate is marked with 'Cw*04 X77'.

Well No. 1 is marked with '1'.

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

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

INTERPRETATION

The interpretation of HLA-Cw*04 SSP subtypings will be influenced by other HLA-Cw alleles, as primer mixes 1, 4, 5, 8, 11, 14, 16 and 20 amplify non-HLA-Cw*04 alleles.

UNIQUELY IDENTIFIED ALLELES

All the HLA-C*04 alleles, i.e. **Cw*0401 to Cw*0427**, recognized by the HLA Nomenclature Committee in April 2007¹ will give rise to unique amplification patterns by the primers in the HLA-Cw*04 subtyping kit.

The HLA-Cw*04 subtyping kit cannot distinguish the Cw*04010101 to Cw*040104 alleles and the Cw*040401 and Cw*040402 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-April-12, release 2.17.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 25 HLA-Cw*04 alleles can be combined in 325 homozygous and heterozygous combinations. 117 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR product generated by primer mix 3 and were not considered in these calculations.

+++++---	--+---+--	-----	0403,0413 = 0406,0418
+++++---	--+-----	-----	0401,0406 = 0403,0404
+++-+--	--+---+--	-----	0407,0413 = 0413,0427
+++-+--	--+-----	-----	0404,0407 = 0404,0427
+++-+---	--+---+--	-----	0401,0413 = 0404,0416 = 0404,0418 =
			0413,0416 = 0413,0418
+++++--+	--+-----	-----	0405,0407 = 0405,0427
+++++--	--+---+--	-----	0405,0416 = 0405,0418
+++++--	--+-----	-----	0401,0405 = 0405,0405
+++++--+	--+-----	-----	0407,0408 = 0408,0427
+++++--	+---+-----	-----	0407,0409N = 0409N,0427
+++++--	+---+-----	-----	0407,0410 = 0410,0427
+++++--	--+---+--	-----	0407,0412 = 0412,0427
+++++--	--+---+--	-----	0407,0418 = 0418,0427
+++++--	--+---+--	-----	0407,0414 = 0414,0427
+++++--	--+---+--	-----	0407,0415 = 0415,0427
+++++--	--+-----	+---+-----	0407,0417 = 0417,0427
+++++--	--+-----	+---+-----	0407,0419 = 0419,0427
+++++--	--+-----	--+-----	0407,0420 = 0420,0427
+++++--	--+-----	--+-----	0407,0421 = 0421,0427
+++++--	--+-----	-----+---	0407,0423 = 0423,0427
+++++--	--+-----	-----+---	0407,0424 = 0424,0427
+++++--	--+-----	-----+---	0407,0425 = 0425,0427
+++++--	--+-----	-----+---	0407,0426 = 0426,0427
+++++--	--+-----	-----	0401,0407 = 0401,0427
+++++--+	--+---+--	-----	0408,0416 = 0408,0418
+++++--+	--+-----	-----	0401,0408 = 0408,0408
+++++---	+---+---	-----	0409N,0416 = 0409N,0418
+++++---	+---+---	-----	0401,0409N = 0409N,0409N
+++++---	--+---+--	-----	0412,0416 = 0412,0418
+++++---	--+---+--	-----	0401,0412 = 0412,0412
+++++---	--+---+--	-----	0414,0416 = 0414,0418
+++++---	--+---+--	-----	0415,0416 = 0415,0418
+++++---	--+---+--	+---+-----	0416,0417 = 0417,0418
+++++---	--+---+--	+---+-----	0416,0419 = 0418,0419
+++++---	--+---+--	--+-----	0416,0420 = 0418,0420
+++++---	--+---+--	--+-----	0416,0421 = 0418,0421
+++++---	--+---+--	-----+---	0416,0423 = 0418,0423
+++++---	--+---+--	-----+---	0416,0424 = 0418,0424
+++++---	--+---+--	-----+---	0416,0425 = 0418,0425
+++++---	--+---+--	-----+---	0416,0426 = 0418,0426
+++++---	--+---+--	-----	0401,0416 = 0401,0418 = 0416,0418 =
			0418,0418
+++++---	--+---+--	-----+---	0401,0415 = 0415,0415 = 0415,0421
+++++---	--+-----	+---+-----	0401,0417 = 0417,0417 = 0417,0421
+++++---	--+-----	+---+-----	0401,0419 = 0419,0419
+++++---	--+-----	--+-----	0401,0420 = 0420,0420

HLA-Cw*04
101.612-12 – licensed for PCR
101.612-12u – not licensed for PCR
Lot No.: **X77**

www.olerup.com

+++-----	--+-----	----+-----	0401,0421 = 0421,0421
+++-----	--+-----	----+-----	0401,0423 = 0423,0423
+++-----	--+-----	-----+--	0401,0424 = 0424,0424
+++-----	--+-----	-----+--	0401,0425 = 0425,0425
+++-----	--+-----	-----+--	0401,0426 = 0426,0426
+++++--+	--+-----	-----+--	0406,0407 = 0406,0427
+++++--+	--+-----	-----+--	0403,0407 = 0403,0427
+++++--+	--+-----	-----+--	0407,0416 = 0416,0427
+++++--+	--+-----	-----+--	0407,0407 = 0407,0427
-++++--	--+-----	-----+--	0404,0413 = 0413,0413

LICENSES

101.612-12 – licensed for PCR.

Notice to purchaser: Limited License.

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

101.612-12u – not licensed for PCR.

Notice to purchaser: Disclaimer of License.

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

101.612-12 and 101.612-12u

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*04 subtyping kit have specificities given in the Specificity and Interpretation Tables of the product insert and in the GenoVision version of the HELMBERG-SCORE™ software.

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

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

The kit is shipped at ambient temperature.

PROTOCOL

DNA EXTRACTION

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

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

IMPORTANT:

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

DNA extracted by other methods, 30 ng/μl.

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

PCR AMPLIFICATION

101.612-12 – licensed for PCR

For one HLA-Cw*04 subtyping, add at room temperature in a 0.5 ml tube:

28 x 2 μl = 56 μl DNA (30 ng/μl)

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

28 x 5 μl = 140 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-H₂O mixture into each of the 24 wells of an HLA-Cw*04 subtyping. **Well No. 1 of the 24 well PCR plate is marked with '1'**. Close the 24 well PCR plate with the provided lids.

101.612-12u – not licensed for PCR

For one HLA-Cw*04 subtyping, add at room temperature in a 0.5 ml tube:

28 x 2 μl = 56 μl DNA (30 ng/μl)

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

2.2 μl *Taq* polymerase (5 units/μl)

28 x 5 μl – 2.2 μl = 137.8 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-*Taq*-H₂O mixture into each of the 24 wells of an HLA-Cw*04 subtyping. **Well No. 1 of the 24 well PCR plate is marked with '1'**. Close the 24 well PCR plate with the provided lids.

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

PCR cycling parameters:

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

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

AGAROSE GEL ELECTROPHORESIS

Prepare a 2% (w/v) agarose gel in 0.5 x TBE buffer. Dissolve the agarose by boiling in a microwave oven. Let the gel solution cool to 60°C. Stain the gel prior to casting with ethidium bromide (10 mg/ml), 5 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*04 SSP subtyping

Specificities and sizes of the PCR products of the 24 primer mixes used for HLA-Cw*04 SSP subtyping

Primer Mix	Approx. Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-Cw*04 alleles	Other amplified HLA Class I alleles ³
1	250 bp	800 bp	04010101-040104, 0403, 0405, 0407-0412, 0415-0418, 0419 ^{weakly} , 0420, 0421, 0423-0427	010201-0118, 020201-020203, 020205, 0204-0215, 0217, 050101-0510, 0512-0516, 06020101-0603, 0605-0615, 0802, 0805, 0807, 0812, 120201-1213, 1215-1217, 1221, 140201-1405, 1407N, 1408, 1508, 160401, 1801-1803
2	220 bp	1070 bp	04010101-040104, 040401-0405, 0407-0409N, 0412-0415, 0417-0421, 0423-0427	
3⁴	145 bp	1070 bp	04010101-040104, 040401-0405, 0408-0415, 0417-0421, 0423-0426	
4	210 bp	800 bp	0403, 0406	0212
5⁵	250 bp	1070 bp	040401-040402, 0406, 0413	0203, 0216, 0218, 0511, 0604, 080101-080102, 0803, 0804, 0806, 0808-0811, 0813, 0814, 1214, 1218, 1220, 1406, 150201-1507,

				1509-1520, 1701-1704
6⁴	95 bp	1070 bp	0405	
7⁴	145 bp	1070 bp	0407, 0427	
8⁵	270 bp	1070 bp	0408	1515
9^{6,7}	280 bp	1070 bp	0409N	
10	220 bp	1070 bp	0410, 0411	
11	180 bp	1070 bp	04010101- 040104, 0403- 0410, 0412- 0421, 0423- 0427	0605
12⁴	125 bp	1070 bp	0411	
13	225 bp	1070 bp	0412	
14⁸	155, 190 bp	1070 bp	0413, 0416, 0418	0708
15⁶	170 bp	1070 bp	0414	
16⁴	130 bp	1070 bp	0415	0305, 0325, 0327
17^{4,6}	90 bp	800 bp	0417	
18	225 bp	1070 bp	0419	
19	155 bp	1070 bp	0420	
20⁴	130 bp	1070 bp	0415, 0417, 0421	030201-030407, 0306-0313, 0315- 0324, 0326, 0328- 0340, 1405
21⁴	85 bp	1070 bp	0423	
22⁴	130 bp	1070 bp	0424	
23⁴	85 bp	1070 bp	0425	
24	170 bp	1070 bp	0426	

¹Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of HLA-Cw*04 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*04 subtyping.

In addition, tubes number 4 and 17 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.

³Due to the sharing of sequence motifs between HLA-Cw alleles some non-HLA-Cw*04 alleles will be amplified by primer mixes 1, 4, 5, 8, 11, 14, 16 and 20.

⁴Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁵Primer mixes 5 and 8 will amplify some B*5802-positive samples.

⁶The primers in primer mixes 9, 15 and 17 have a tendency of giving rise to nonspecific amplifications.

⁷The specific PCR product generated by the primers in primer mix 9 may be less intense than the other Cw*04 specific PCR products.

⁸Primer mix 14: Specific PCR fragment of 155 bp in the Cw*0416 allele. Specific PCR fragment of 190 bp in the Cw*0413, Cw*0418 and Cw*0708 alleles.

INTERPRETATION TABLE												
HLA-Cw*04 SSP subtyping												
Amplification patterns of the Cw*0401 to Cw*0427 alleles												
	Tube ⁵											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	250	220	145	210	250	95	145	270	280	220	180	125
PCR product												
Length of int.	800	1070	1070	800	1070	1070	1070	1070	1070	1070	1070	1070
pos. control ¹												
5'-primer(s) ²	2nd 	112	112	118	2nd 	98	112	2nd 	1017	112	201	218
	5'-CCA ^{3'}	5'-CCT ^{3'}	5'-CCT ^{3'}	5'-CCA ^{3'}	5'-CCA ^{3'}	5'-CTC ^{3'}	5'-CCT ^{3'}	5'-CCA ^{3'}	5'-gTg ^{3'}	5'-CCT ^{3'}	5'-CCA ^{3'}	5'-ggA ^{3'}
3'-primer(s) ³	539	289	218	289	539	154	218	559	1092	289	341	302
	5'-TCC ^{3'}	5'-AgC ^{3'}	5'-gCT ^{3'}	5'-AgC ^{3'}	5'-TCA ^{3'}	5'-CAG ^{3'}	5'-gCg ^{3'}	5'-CAG ^{3'}	5'-TTA ^{3'}	5'-AgT ^{3'}	5'-CgT ^{3'}	5'-ggC ^{3'}
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-Cw allele ⁴												
*04010101-040104	+	+	+								+	
*0403	+			+							+	
*040401-040402		+	+		+						+	
*0405	+	+	+			+					+	
*0406				+	+						+	
*0407	+	+					+				+	
*0408	+	+	+					+			+	
*0409N	+	+	+						+		+	
*0410	+		+							+	+	
*0411	+		+							+		+
*0412	+	+	+								+	
*0413		+	+		+						+	
*0414		+	+								+	
*0415	+	+	+								+	
*0416	+										+	
*0417	+	+	+								+	
*0418	+	+	+								+	
*0419	W	+	+								+	
*0420	+	+	+								+	
*0421	+	+	+								+	
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE												
HLA-Cw*04 SSP subtyping												
Amplification patterns of the Cw*0401 to Cw*0427 alleles												
Tube ⁵												
13	14	15	16	17	18	19	20	21	22	23	24	
225	155	170	130	90	225	155	130	85	130	85	170	Length of spec. PCR product
	190											
1070	1070	1070	1070	800	1070	1070	1070	1070	1070	1070	1070	Length of int. pos. control ¹
112	105	412	369	2 nd I	368	347	368	368	127	172	89	5'-primer ²
5'-CCT ^{3'}	5'-gCT ^{3'}	5'-ATA ^{3'}	5'-TAC ^{3'}	5'-CCA ^{3'}	5'-gTT ^{3'}	5'-gTA ^{3'}	5'-gTA ^{3'}	5'-gTT ^{3'}	5'-ggA ^{3'}	5'-TCC ^{3'}	5'-gAT ^{3'}	
	368											
	5'-gTT ^{3'}											
295	218	539	459	379	550	459	459	412	218	218	218	3'-primer ³
5'-TCC ^{3'}	5'-gCT ^{3'}	5'-TCT ^{3'}	5'-AgA ^{3'}	5'-CAC ^{3'}	5'-CAT ^{3'}	5'-AgA ^{3'}	5'-AgA ^{3'}	5'-gTC ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	
	514											
	5'-CTT ^{3'}											
	527											
	5'-CCg ^{3'}											
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.
												HLA-Cw allele ⁴
												*04010101
												*0403
												*040401
												*0405
												*0406
												*0407
												*0408
												*0409N
												*0410
												*0411
+												*0412
	+											*0413
		+										*0414
			+				+					*0415
	+											*0416
				+			+					*0417
	+											*0418
					+							*0419
						+						*0420
							+					*0421
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.

Length of spec.	250	220	145	210	250	95	145	270	280	220	180	125
PCR product												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
*0423	+	+	+								+	
*0424	+	+	+								+	
*0425	+	+	+								+	
*0426	+	+	+								+	
*0427	+	+					+					
*010201-0118, 020201-020203, 020205, 0204-0211, 0213-0215, 0217, 050101-0510, 0512-0516, 06020101-0603, 0606-0616N, 0802, 0805, 0807, 0812, 120201-1213, 1215-1217, 1221, 140201-1404, 1407N, 1408, 1508, 160401, 1801-1803	+											
*0203, 0216, 0218, 0511, 0604, 080101-080102, 0803, 0804, 0806, 0808-0811, 0813, 0814, 1214, 1218, 1220, 1406, 150201-1507, 1509-1514, 1516-1520, 1701-1704					+							
*0212	+			+								
*030201-030407, 0306-0313, 0315-0324, 0326, 0328-0340												
*0305, 0325, 0327												
*0605	+										+	
*0708												
*1405	+											
*1515					+			+				
HLA-Cw allele ⁴												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12

HLA-Cw*04
 101.612-12 – licensed for PCR
 101.612-12u – not licensed for PCR
 Lot No.: **X77**

225	155	170	130	90	225	155	130	85	130	85	170	Length of spec. PCR product Tube No.
13	14	15	16	17	18	19	20	21	22	23	24	
								+				*0423
									+			*0424
										+		*0425
											+	*0426
												*0427
												*010201-0118, 020201-020203, 020205, 0204-0211, 0213-0215, 0217, 050101-0510, 0512-0516, 06020101-0603, 0606-0616N, 0802, 0805, 0807, 0812, 120201-1213, 1215-1217, 1221, 140201-1404, 1407N, 1408, 1508, 160401, 1801-1803
												*0203, 0216, 0218, 0511, 0604, 080101-080102, 0803, 0804, 0806, 0808-0811, 0813, 0814, 1214, 1218, 1220, 1406, 150201-1507, 1509-1514, 1516-1520, 1701-1704
												*0212
							+					*030201-030407, 0306-0313, 0315-0324, 0326, 0328-0340
			+									*0305, 0325, 0327
												*0605
	+											*0708
							+					
												*1515
												HLA-Cw allele ⁴
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.



¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most tubes, or a band of 800 base pairs, for some tubes.

Tube number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-Cw*04 subtyping. .

In addition, tubes number 4 and 17 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

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

²The nucleotide position, in the 2nd, 3rd or 6th, exon or in 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, 3rd or 7th exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as in *Tissue Antigens* 1998, **51:II**, 417-466. The sequence of the 3 terminal nucleotides of the primer is given.

⁴The nucleotide sequence of the HLA-Cw*0402 allele has been shown to be identical to Cw*04010101.

The nucleotide sequence of the HLA-Cw*0422 allele has been shown to be identical to Cw*0421.

Primer mixes 5 and 8 will amplify some B*5802-positive samples.

⁵Primer mix 14: Specific PCR fragment of 155 bp in the Cw*0416 allele. Specific PCR fragment of 190 bp in the Cw*0413, Cw*0418 and Cw*0708 alleles.

CELL LINE VALIDATION SHEET																				
HLA-Cw*04 SSP subtyping kit																				
				Tube																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Prod. No.:	200618401	200618402	200618403	200618404	200618405	200618406	200618407	200618408	200618409	200733710	200618411	200618412	200733713	200733714	200618415	200618416
		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	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	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-



HLA-Cw*04
 101.612-12 – licensed for PCR
 101.612-12u – not licensed for PCR
 Lot No.: **X77**

CELL LINE VALIDATION SHEET													
HLA-Cw*04 SSP subtyping kit													
					Tube								
					17	18	19	20	21	22	23	24	
					Prod. No.:	200733717	200733718	200733719	200733720	200733721	200733722	200733723	200733724
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	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*04 SSP

Product number: 101.612-12 – licensed for PCR
101.612-12u – not licensed for PCR
Lot number: X77
Expiry date: 2009-April-01
Number of tests: 12
Number of tubes per test: 24

Tube specifications:

Tube No.	Production No.	Tube No.	Production No.	Tube No.	Production No.
1	2006-184-01	9	2006-184-09	17	2007-337-17
2	2006-184-02	10	2007-337-10	18	2007-337-18
3	2006-184-03	11	2006-184-11	19	2007-337-19
4	2006-184-04	12	2006-184-12	20	2007-337-20
5	2006-184-05	13	2007-337-13	21	2007-337-21
6	2006-184-06	14	2007-337-14	22	2007-337-22
7	2006-184-07	15	2006-184-15	23	2007-337-23
8	2006-184-08	16	2006-184-16	24	2007-337-24

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 6, 7, 8, 10, 12, 13, 15, 17 to 19 and 21 to 24 were available. The specificity of the primers in primer solutions 7, 8, 10, 12, 15, 17 and 21 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 6, 13 and 18 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 19, 22, 23 and 24 it was only possible to test the 3'-primers, the 5'-primers were not possible to test.

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

Date of approval: 2007-June-12

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: Olerup SSP™ HLA-Cw*04
Product number: 101.612-12, 101.612-12u
Lot number: X77

Intended use: HLA-Cw*04 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 9001:2003, ISO 17025:1999 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex III.

The Technical Construction 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-June-12

Olle Olerup
Managing Director

HLA-Cw*04
101.612-12 – licensed for PCR
101.612-12u – not licensed for PCR
Lot No.: **X77**

21

www.olerup.com



HLA-Cw*04
101.612-12 – licensed for PCR
101.612-12u – not licensed for PCR
Lot No.: **X77**

22

www.olerup.com

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*04
101.612-12 – licensed for PCR
101.612-12u – not licensed for PCR
Lot No.: **X77**

24

www.olerup.com

ADDRESSES:

Manufacturer:

Olerup SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

Tel: +46-8-717 88 27

Fax: +46-8-717 88 18

E-mail: info-ssp@olerup.com

Web page: <http://www.olerup.com>

Distributed by:

Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.

Tel: +43-1-710 15 00

Fax: +43-1-710 15 00 10

E-mail: support-at@olerup.com

Web page: <http://www.olerup.com>

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382

Tel: 1-877-OLERUP1

Fax: 610-344-7989

E-mail: info.us@olerup.com

Web page: <http://www.olerup.com>

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