

## Olerup SSP™ HLA-Cw\*07

**Product number:** 101.615-24/04 – licensed for PCR  
101.615-24u/04u - not licensed for PCR  
**Lot number:** X93  
**Expiry date:** 2009-June-01  
**Number of tests:** 24 tests – Product No. 101.615-24/24u  
4 tests – Product No. 101.615-04/04u  
**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. X93.**

### CHANGES COMPARED TO THE PREVIOUS OLERUP SSP™ HLA-Cw\*07 LOT

The HLA-Cw\*07 specificity and interpretation tables have been updated for the HLA-Cw alleles described since the previous Olerup SSP™ HLA-Cw\*07 lot was made (Lot No. V36).

Two tubes have been added to the HLA-Cw\*07 kit,  
wells **31 and 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
2	Modified	-	Primer modified for the Cw*0743 allele.
6	Modified	-	Increased yield of specific PCR product.
8	Added	Added	Primer pair added for the Cw*0745 allele.
17	Modified	-	Increased yield of specific PCR product.
20	Added	-	Primers added for the Cw*0744 and 0748 alleles.
22	Modified	-	Increased specificity of specific primer pair.
23	Modified	-	Increased yield of specific PCR product.
25	Added	Added	Primer pair added for the Cw*0746 allele.
28	Added	-	Primer added for the Cw*0747 allele.
29	Added	Added	Primer pair added for the Cw*0742 allele.
31	New	New	New primer pair for the Cw*0739 and 0740 alleles.
32	New	New	New primer pair for the Cw*0749 allele.

## PRODUCT DESCRIPTION

### HLA-Cw\*07 SSP subtyping

#### CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-Cw\*0701 to HLA-Cw\*0749 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.

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 30 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 'HLA-Cw\*07 X93'.

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\*07 SSP subtypings will be influenced by the Cw\*0214 allele, two Cw\*03 alleles, three Cw\*04 alleles, most Cw\*05 alleles, most Cw\*06 alleles, most Cw\*08 alleles and the Cw\*18 alleles when present on the other haplotype. In addition, the A\*0284 allele will be amplified by primer mix 8, the A\*2910 allele will be amplified by primer mix 12, the B\*0806 and B\*0817 alleles will be amplified by primer mix 32, the B\*2736 allele will be amplified by primer mix 7, the B\*4060 allele by primer mixes 4, 18 and 30 and the B\*5608 and B\*5614 alleles will be weakly amplified by primer mix 25.

### UNIQUELY IDENTIFIED ALLELES

All the HLA-Cw\*07 alleles, i.e. **Cw\*0701 to Cw\*0749**, recognized by the HLA Nomenclature Committee in July 2007<sup>1</sup> will give rise to unique amplification patterns by the primers in the HLA-Cw\*07 subtyping kit.

The HLA-Cw\*07 subtyping kit cannot distinguish the Cw\*070101 to Cw\*070104 and Cw\*070106 alleles, the Cw\*07020101 and Cw\*07020103 alleles or the Cw\*070401 and Cw\*070402 alleles.

<sup>1</sup>**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-July-09, release 2.18.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

### RESOLUTION IN HOMO- AND HETEROZYGOTES

The 49 HLA-Cw\*07 alleles give rise to 51 amplification patterns, as the Cw\*070105 and Cw\*070107 alleles have different amplification patterns than the other Cw\*0701xx alleles, that can be combined in 1 326 homozygous and heterozygous combinations. 418 of these genotypes do not give rise to unique amplification patterns. (The different lengths of the specific PCR products in lanes 8, 9, 11, 19, 20, 23, 25 to 27 were not considered in these calculations.)

++++----	+-----+	-----	-----+	0709,0710 = 0724,0749
++++----	+-----+	-----	-----+	0709,0713 = 0728,0749
++++----	+-----+	-----	-----+	0709,0733N = 0726,0749
++++----	+-----+	-----	-----+	0709,0748 = 0744,0749
++++----	+-----+	-----	-----+	0709,0723 = 0721,0749
++++----	+-----+	-----	-----+	0709,0725 = 0722,0749
++++----	+-----+	-----	+-----+	0709,0746 = 0730,0749
++++----	+-----+	-----	-----+	070107,0749 = 0709,0737
++++----	+-----+	-----	-----+	0709,0747 = 0735,0749
++++----	+-----+	-----	-----+	0709,0742 = 0736,0749
++++----	+-----+	-----	-----+	0709,0739 = 0740,0749
++++----	+-----+	-----	-----+	0701,0749 = 0702,0709 = 0709,0729 = 0709,0749
++++----	---+---+	-----	-----	0710,0728 = 0713,0724
++++----	---+---+	-----	-----	0710,0726 = 0724,0733N
++++----	---+---+	-----	-----	0710,0744 = 0724,0748
++++----	---+---+	-----	-----	0710,0718 = 0719,0743
++++----	---+---+	-----	-----	0710,0721 = 0723,0724
++++----	---+---+	-----	-----	0710,0722 = 0724,0725
++++----	---+---+	-----	+-----	0710,0730 = 0724,0746
++++----	---+---+	-----	---+---	070107,0710 = 0724,0737
++++----	---+---+	-----	---+---	0710,0735 = 0724,0747
++++----	---+---+	-----	---+---	0710,0736 = 0724,0742
++++----	---+---+	-----	---+---	0710,0740 = 0724,0739
++++----	---+---+	-----	-----	0701,0710 = 0702,0724 = 0710,0724
++++----	-----+	---+---	-----	0713,0726 = 0728,0733N
++++----	-----+	---+---	-----	0713,0744 = 0728,0748
++++----	-----+	---+---	-----	0713,0721 = 0723,0728
++++----	-----+	---+---	-----	0713,0722 = 0725,0728
++++----	-----+	---+---	+-----	0713,0730 = 0728,0746
++++----	-----+	---+---	---+---	070107,0713 = 0728,0737
++++----	-----+	---+---	---+---	0713,0735 = 0728,0747
++++----	-----+	---+---	---+---	0713,0736 = 0728,0742
++++----	-----+	---+---	---+---	0713,0740 = 0728,0739
++++----	-----+	---+---	-----	0701,0713 = 0702,0728 = 0713,0728

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++++----- +-----+ --++----- ----- 0726,0748 = 0733N,0744  
++++----- +-----+ --+-----+----- 0721,0733N = 0723,0726  
++++----- +-----+ --+-----+----- 0722,0733N = 0725,0726  
++++----- +-----+ --+----- +----- 0726,0746 = 0730,0733N  
++++----- +-----+ --+----- --+----- 070107,0733N = 0726,0737  
++++----- +-----+ --+----- ---+----- 0726,0747 = 0733N,0735  
++++----- +-----+ --+----- ----+----- 0726,0742 = 0733N,0736  
++++----- +-----+ --+----- -----+ 0726,0739 = 0733N,0740  
++++----- +-----+ --+----- ----- 0701,0733N = 0702,0726 = 0726,0733N  
++++----- +-----+ ---+-----+----- 0721,0748 = 0723,0744  
++++----- +-----+ ---+-----+----- 0722,0748 = 0725,0744  
++++----- +-----+ ---+-----+----- 0730,0748 = 0744,0746  
++++----- +-----+ ---+----- --+----- 070107,0748 = 0737,0744  
++++----- +-----+ ---+----- ---+----- 0735,0748 = 0744,0747  
++++----- +-----+ ---+----- ----+----- 0736,0748 = 0742,0744  
++++----- +-----+ ---+----- -----+ 0739,0744 = 0740,0748  
++++----- +-----+ ---+----- ----- 0701,0748 = 0702,0744 = 0744,0748  
++++----- +-----+ ---+----- -+----- 0718,0732N = 0719,0731  
++++----- +-----+ ---+----- --+----- 0718,0737 = 0719,0734  
++++----- +-----+ ---+----- ----- 0702,0718 = 0719,0727  
++++----- +-----+ -----++----- 0721,0725 = 0722,0723  
++++----- +-----+ -----+----- 0721,0746 = 0723,0730  
++++----- +-----+ -----+----- --+----- 070107,0723 = 0721,0737  
++++----- +-----+ -----+----- ---+----- 0721,0747 = 0723,0735  
++++----- +-----+ -----+----- ----+----- 0721,0742 = 0723,0736  
++++----- +-----+ -----+----- -----+ 0721,0739 = 0723,0740  
++++----- +-----+ -----+----- ----- 0701,0723 = 0702,0721 = 0721,0723  
++++----- +-----+ -----+----- +----- 0722,0746 = 0725,0730  
++++----- +-----+ -----+----- --+----- 070107,0725 = 0722,0737  
++++----- +-----+ -----+----- ---+----- 0722,0747 = 0725,0735  
++++----- +-----+ -----+----- ----+----- 0722,0742 = 0725,0736  
++++----- +-----+ -----+----- -----+ 0722,0739 = 0725,0740  
++++----- +-----+ -----+----- ----- 0701,0725 = 0702,0722 = 0722,0725  
++++----- +-----+ -----+----- +-+----- 070107,0746 = 0730,0737  
++++----- +-----+ -----+----- +--+----- 0730,0747 = 0735,0746  
++++----- +-----+ -----+----- +---+----- 0730,0742 = 0736,0746  
++++----- +-----+ -----+----- +-----+ 0730,0739 = 0740,0746  
++++----- +-----+ -----+----- +----- 0701,0746 = 0702,0730 = 0730,0746  
++++----- +-----+ -----+----- --++----- 070107,0747 = 0735,0737  
++++----- +-----+ -----+----- --+----- 070107,0742 = 0736,0737  
++++----- +-----+ -----+----- --+-----+ 070107,0739 = 0737,0740  
++++----- +-----+ -----+----- --+----- 0701,0737 = 070107,0702 = 070107,0737  
++++----- +-----+ -----+----- ---++----- 0735,0742 = 0736,0747  
++++----- +-----+ -----+----- ---+-----+ 0735,0739 = 0740,0747  
++++----- +-----+ -----+----- ---+----- 0701,0747 = 0702,0735 = 0735,0747  
++++----- +-----+ -----+----- ----+-----+ 0736,0739 = 0740,0742  
++++----- +-----+ -----+----- -----+ 0701,0742 = 0702,0736 = 0736,0742  
++++----- +-----+ -----+----- -----+ 0701,0739 = 0702,0740 = 0739,0740  
++++----- +-----+ ---+-----+----- 0716,0719 = 0719,0726  
++++----- +-----+ ---+-----+----- 0701,0719 = 0718,0719  
++++----- +-----+ ---+-----+----- 0701,0703 = 0703,0744  
++++----- +-----+ ---+-----+----- 0701,0705 = 070105,0705  
++++----- +-----+ ---+-----+----- 0706,0727 = 0706,0745 = 0718,0745  
++++----- +-----+ ---+-----+----- 0706,0716 = 0706,0726  
++++----- +-----+ ---+-----+----- 0701,0706 = 070105,0706 = 0706,0706 =  
0706,0718  
++++----- +-----+ --+-----+-----+ 0701,0707 = 070105,0707 = 0707,0709 =  
0707,0726 = 0709,0716 = 0709,0726  
++++----- +-----+ -----+-----+-----+ 0701,0709 = 070105,0709 = 0709,0709



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+++----- --+-----+ ---+----- ----- 0717,0724 = 0743,0744  
+++----- --+-----+ ----- --+----- 070107,0743 = 0724,0734  
+++----- --+-----+ ----- ----- 0701,0743 = 070105,0743 = 0724,0727 =  
0724,0743  
+++----- --+----- --+----- ----- 0716,0724 = 0724,0726  
+++----- --+----- ----- ----- 0701,0724 = 070105,0724 = 0724,0724  
+++----- -----+-- --+----- ----- 0716,0728 = 0726,0728  
+++----- -----+-- ----- ----- 0701,0728 = 070105,0728 = 0728,0728  
+++----- -----++ ----- --+----- 0701,0714 = 070105,0714 = 070107,0714  
+++----- -----+ ---+----- --+----- 070107,0717 = 0734,0744  
+++----- -----+ ---+----- ----- 0701,0717 = 0717,0744 = 0727,0744  
+++----- -----+ ----- -+----- 0701,0731 = 070105,0731  
+++----- -----+ ----- --+----- 0701,0734 = 070105,0734 = 070107,0727 =  
070107,0734  
+++----- -----+ ----- ----- 0701,0727 = 070105,0727  
+++----- ----- --+----- ----- 0716,0744 = 0726,0744  
+++----- ----- --+----- ----- 0716,0718 = 0718,0726  
+++----- ----- --+----- ----- 0716,0720 = 0720,0726  
+++----- ----- --+----- ----- 0716,0721 = 0721,0726  
+++----- ----- --+-----+ ----- 0716,0722 = 0722,0726  
+++----- ----- --+----- +----- 0716,0730 = 0726,0730  
+++----- ----- --+----- --+----- 070107,0716 = 070107,0726  
+++----- ----- --+----- --+----- 0716,0735 = 0726,0735  
+++----- ----- --+----- -----+----- 0716,0736 = 0726,0736  
+++----- ----- --+----- -----+----- 0716,0740 = 0726,0740  
+++----- ----- --+----- ----- 0701,0716 = 0701,0726 = 070105,0716 =  
070105,0726 = 0716,0726 = 0726,0726  
+++----- ----- --+----- ----- 0701,0744 = 070105,0744 = 0744,0744  
+++----- ----- -----+----- ----- 0701,0718 = 070105,0718 = 0718,0718  
+++----- ----- -----+----- ----- 0701,0720 = 070105,0720 = 0720,0720  
+++----- ----- -----+----- ----- 0701,0721 = 070105,0721 = 0721,0721  
+++----- ----- -----+----- ----- 0701,0722 = 070105,0722 = 0722,0722  
+++----- ----- -----+----- +----- 0701,0730 = 070105,0730 = 0730,0730  
+++----- ----- ----- --+----- 0701,070107 = 070105,070107 =  
070107,070107  
+++----- ----- ----- --+----- 0701,0735 = 070105,0735 = 0735,0735  
+++----- ----- ----- --+----- 0701,0736 = 070105,0736 = 0736,0736  
+++----- ----- ----- -----+----- 0701,0740 = 070105,0740 = 0740,0740  
+++----- ----- ----- ----- 0701,0701 = 0701,070105  
+++----- +-----+ --+----- -----+ 0702,0707 = 0707,0729 = 0707,0733N =  
0707,0749 = 0716,0749  
+++----- --+-----+ --+----- ----- 0710,0716 = 0733N,0743  
+++----- --+-----+ ----- -+----- 0710,0731 = 0732N,0743  
+++----- --+-----+ ----- --+----- 0710,0734 = 0737,0743  
+++----- --+-----+ ----- ----- 0702,0743 = 0710,0727 = 0710,0743  
+++----- -----++ ----- --+----- 0702,0714 = 0714,0737  
+++----- -----++ --+----- -+----- 0716,0732N = 0731,0733N  
+++----- -----+ --+----- --+----- 0716,0737 = 0733N,0734  
+++----- -----+ --+----- ----- 0702,0716 = 0716,0733N = 0727,0733N  
+++----- -----+ ----- --+----- 0731,0737 = 0732N,0734  
+++----- -----+ ----- -+----- 0702,0731 = 0727,0732N = 0731,0732N  
+++----- -----+ ----- --+----- 0702,0734 = 0727,0737 = 0734,0737  
+++-----+-----+ ----- ----- 0705,0705 = 0705,0727  
+++----- +----- --+----- -----+ 0707,0707 = 0707,0716  
+++----- --+-----+ ----- ----- 0727,0743 = 0743,0743  
+++----- -----++ ----- --+----- 0714,0714 = 0714,0727 = 0714,0734  
+++----- -----+ ----- -+----- 0727,0731 = 0731,0731  
+++----- -----+ ----- --+----- 0727,0734 = 0734,0734  
+++----- -----+ ----- --+----- 0717,0719 = 0719,0748

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+--+----- -+----- -+----- -+----- 070105,0719 = 0719,0719  
+--+----- -+----- -+----- -+----- 0702,0703 = 0703,0748  
+--+----- +-----+ -+----- -+-----+ 0717,0749 = 0748,0749  
+--+----- +-----+ -+----- -+----- 0717,0729 = 0729,0748  
+--+----- +-----+ -+----- -+-----+ 0702,0749 = 0729,0749 = 0749,0749  
+--+----- +-----+ -+----- -+----- 0702,0729 = 0729,0729  
+--+----- -+-----+ -+----- -+----- 0710,0717 = 0710,0748  
+--+----- -+-----+ -+----- -+----- 0702,0710 = 0710,0710  
+--+----- -+-----+ -+----- -+----- 0713,0717 = 0713,0748  
+--+----- -+-----+ -+----- -+----- 0702,0713 = 0713,0713  
+--+----- -+-----+ -+----- -+----- 0715,0717 = 0715,0748  
+--+----- -+-----+ -+----- -+----- 0702,0715 = 0715,0715  
+--+----- -+-----+ -+----- -+----- 0717,0733N = 0733N,0748  
+--+----- -+-----+ -+----- -+----- 0702,0733N = 0733N,0733N  
+--+----- -+-----+ -+----- -+----- 0717,0723 = 0723,0748  
+--+----- -+-----+ -+----- -+----- 0717,0725 = 0725,0748  
+--+----- -+-----+ -+----- -+----- 0717,0746 = 0746,0748  
+--+----- -+-----+ -+----- -+----- 0717,0732N = 0732N,0748  
+--+----- -+-----+ -+----- -+----- 0717,0737 = 0737,0748  
+--+----- -+-----+ -+----- -+----- 0717,0747 = 0747,0748  
+--+----- -+-----+ -+----- -+----- 0717,0742 = 0742,0748  
+--+----- -+-----+ -+----- -+----- 0717,0738 = 0738,0748  
+--+----- -+-----+ -+----- -+----- 0717,0739 = 0739,0748  
+--+----- -+-----+ -+----- -+----- 0702,0717 = 0702,0748 = 0717,0748 =  
0748,0748  
+--+----- -+-----+ -+----- -+----- 0702,0723 = 0723,0723  
+--+----- -+-----+ -+----- -+----- 0702,0725 = 0725,0725  
+--+----- -+-----+ -+----- -+----- 0702,0746 = 0746,0746  
+--+----- -+-----+ -+----- -+----- 0702,0732N = 0732N,0732N  
+--+----- -+-----+ -+----- -+----- 0702,0737 = 0737,0737  
+--+----- -+-----+ -+----- -+----- 0702,0747 = 0747,0747  
+--+----- -+-----+ -+----- -+----- 0702,0742 = 0742,0742  
+--+----- -+-----+ -+----- -+----- 0702,0738 = 0738,0738  
+--+----- -+-----+ -+----- -+----- 0702,0739 = 0739,0739  
+--+----- -+-----+ -+----- -+----- 0703,0703 = 0703,0717  
+--+----- -+-----+ -+----- -+----- 0711,0712 = 0711,0741  
+--+----- -+-----+ -+----- -+----- 0704,0712 = 0704,0741  
+--+----- -+-----+ -+----- -+----- 0712,0712 = 0712,0741

0701 = 070101-070104 +070106

## LICENSES

### 101.615-24/04 – licensed for PCR.

#### Notice to purchaser: Limited License.

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### 101.615-24u/04u – not licensed for PCR.

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### 101.615-24/04 and 101.615-24u/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-Cw\*07 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 dH<sub>2</sub>O.

### 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

#### **101.615-24/04 – licensed for PCR**

For one HLA-Cw\*07 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 dH<sub>2</sub>O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-H<sub>2</sub>O mixture into each of the 32 wells of a HLA-Cw\*07 subtyping. **Well No. 1 of the 32 well PCR plate is marked with '1'**. Close the 32 well PCR plate with the provided lids.

#### **101.615-24u/04u – not licensed for PCR**

For one HLA-Cw\*07 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 dH<sub>2</sub>O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-*Taq*-H<sub>2</sub>O mixture into each of the 32 wells of an HLA-Cw\*07 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 $\mu$ l SSP reaction
nucleotides	final concentration of each dNTP is 200 $\mu$ M
PCR buffer	final concentrations: 50 mM KCl, 1.5 mM MgCl <sub>2</sub> , 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 $\mu$ 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 $\mu$ M
PCR buffer	final concentrations: 50 mM KCl, 1.5 mM MgCl <sub>2</sub> , 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 $\mu$ 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  $-20^{\circ}\text{C}$ , 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\*07 SSP subtyping

Specificities and sizes of the PCR products of the 32 primer mixes used for HLA-Cw\*07 SSP subtyping

Primer Mix	Approx. size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-Cw*07 alleles <sup>3</sup>	Other amplified HLA-Class I alleles <sup>4</sup>
<b>1<sup>5</sup></b>	80 bp	<b>800 bp</b>	070101-0705, 0706 <sup>weakly</sup> , 0707-0710 <sup>?</sup> , 0712-0717 <sup>?</sup> , 0718 <sup>weakly</sup> , 0719 <sup>weakly</sup> , 0720-0725 <sup>?</sup> , 0726, 0727-0729 <sup>?</sup> , 0730, 0731 <sup>?</sup> , 0732N, 0733N-0735 <sup>?</sup> , 0736, 0737-0741 <sup>?</sup> , 0742, 0743-0748 <sup>?</sup> , 0749	
<b>2</b>	290 bp	<b>800 bp</b>	070101-070104, 070106-070107, 0705-0707, 0709, 0714, 0716, 0718, 0720-0722, 0724, 0726-0728, 0730, 0731, 0734-0736, 0740, 0743, 0744	0517
<b>3<sup>5</sup></b>	110 bp	<b>800 bp</b>	070101-070107, 0706, 0709, 0718-0722, 0724, 0726, 0728, 0730, 0735, 0736, 0740, 0744	
<b>4</b>	185 bp	1070 bp	07020101-07020103, 0710, 0713, 0715, 0719, 0723, 0725, 0729, 0732N, 0733N, 0737-0739, 0742, 0746-0749	<b>B*4060</b>
<b>5<sup>6</sup></b>	245 bp	<b>800 bp</b>	0703	
<b>6<sup>5</sup></b>	100 bp	1070 bp	070401-070402, 0711, 0712	
<b>7</b>	190 bp	1070 bp	0705	<b>B*2736</b>
<b>8<sup>6,7,8</sup></b>	285, 645 bp	1070 bp	0706, 0745	<b>A*0284</b>

<b>9<sup>6,9</sup></b>	240, 425 bp	<b>800 bp</b>	0707, 0709, 0729, 0749	1801-1803
<b>10</b>	185 bp	1070 bp	0708	
<b>11<sup>10</sup></b>	275, 305 bp	<b>800 bp</b>	0710, 0724, 0743	
<b>12<sup>5</sup></b>	130 bp	1070 bp	0712, 0741	050101-050103, 0503-0508, 0510-0516, 080101-0806, 0808-0814, <b>A*2910</b>
<b>13<sup>5,7</sup></b>	80 bp	1070 bp	0711	
<b>14</b>	240 bp	<b>800 bp</b>	0713, 0728	0517
<b>15</b>	290 bp	<b>800 bp</b>	0714	
<b>16</b>	395 bp	<b>800 bp</b>	07020101-0705, 0708, 0710-0715, 0717, 0723, 0725, 0727, 0729, 0731-0734, 0737-0739, 0741-0743, 0745-0749	1801-1803
<b>17<sup>5</sup></b>	130 bp	1070 bp	070401-070402, 0711	0807
<b>18</b>	230 bp	<b>800 bp</b>	0715	<b>B*4060</b>
<b>19<sup>11</sup></b>	220, 465 bp	<b>800 bp</b>	0707, 0716, 0726, 0733N	
<b>20<sup>5,12</sup></b>	145, 170 bp	1070 bp	0703, 0717, 0744, 0748	0517
<b>21<sup>7</sup></b>	225 bp	<b>800 bp</b>	0706, 0718, 0719	
<b>22<sup>6</sup></b>	250 bp	<b>800 bp</b>	0720	0315, 0332, 0403, 0406, 0416
<b>23<sup>13</sup></b>	155, 185 bp	1070 bp	0721, 0723	
<b>24<sup>5</sup></b>	130 bp	1070 bp	0722, 0725	
<b>25<sup>5,14</sup></b>	105, 150 bp	1070 bp	0730, 0746	<b>B*5608<sup>weakly</sup>, B*5614<sup>weakly</sup></b>
<b>26<sup>5,15</sup></b>	100, 300 bp	1070 bp	0731, 0732N	
<b>27<sup>5,16</sup></b>	120, 290 bp	1070 bp	070107, 0714, 0734, 0737	
<b>28</b>	260 bp	<b>800 bp</b>	0735, 0747	
<b>29</b>	255 bp	1070 bp	0736, 0742	
<b>30</b>	275 bp	1070 bp	0738	<b>B*4060</b>
<b>31</b>	415 bp	1070 bp	0739, 0740	
<b>32</b>	245 bp	1070 bp	0707, 0709, 0749	0214, 06020101-060202, 0604-0616N, 1801-1803, <b>B*0806, B*0817</b>

<sup>1</sup> 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\*07 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, e.g. the 3'-primer in primer mixes 13 and 21.

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.

<sup>2</sup> The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most tubes, or a band of 800 base pairs, for some tubes.

Tube number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-Cw\*07 subtyping.

In addition, tubes number 2, 3, 5, 9, 11, 14, 15, 16, 18, 19, 21, 22 and 28 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.

<sup>3</sup> For several HLA-Cw\*07 alleles 1<sup>st</sup>, 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> exon nucleotide sequences are not available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. We assume that unknown sequences in the 1<sup>st</sup>, 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> exons are conserved within allelic groups.

<sup>4</sup> Due to the sharing of sequence motifs between HLA-Cw alleles non-HLA-Cw\*07 alleles will be amplified by primer mixes 2, 9, 12, 14, 16, 17, 20, 22 and 32. In addition, the A\*0284 allele will be amplified by primer mix 8, the A\*2910 allele will be amplified by primer mix 12, the B\*0806 and B\*0817 alleles will be amplified by primer mix 32, the B\*2736 allele will be amplified by primer mix 7, the B\*4060 allele by primer mixes 4, 18 and 30 and the B\*5608 and B\*5614 alleles will be weakly amplified by primer mix 25.

<sup>5</sup> Short specific PCR fragments are less intense and not as sharp as longer specific bands.

<sup>6</sup> Primer mixes 5, 8, 9 and 22 have a tendency of primer oligomer formation.

<sup>7</sup> Primer mixes 8, 13 and 21 have a tendency of giving rise to nonspecific amplifications.

<sup>8</sup> Primer mix 8: Specific PCR fragment of 285 bp in the Cw\*0745 and A\*0284 alleles. Specific PCR fragment of 645 bp in the Cw\*0706 allele.

<sup>9</sup> Primer mix 9: Specific PCR fragment of 240 bp in the Cw\*0729 allele. Specific PCR fragment of 425 bp in the Cw\*0707, 0709 and 0749 and the Cw\*1801-1802 alleles.

<sup>10</sup> Primer mix 11: Specific PCR fragment of 275 bp in the Cw\*0724 allele. Specific PCR fragment of 305 bp in the Cw\*0710 and 0743 alleles.

<sup>11</sup> Primer mix 19: Specific PCR fragment of 220 bp in the Cw\*0726 and 0733N alleles. Specific PCR fragment of 465 bp in the Cw\*0707 and 0716 alleles.

<sup>12</sup> Primer mix 20: Specific PCR fragment of 145 bp in the Cw\*0703 and 0717 and Cw\*0517 alleles. Specific PCR fragment of 170 bp in the Cw\*0744 and 0748 alleles.

<sup>13</sup> Primer mix 23: Specific PCR fragment of 155 bp in the Cw\*0723 allele. Specific PCR fragment of 185 bp in the Cw\*0721 allele.

<sup>14</sup> Primer mix 25: Specific PCR fragment of 105 bp in the Cw\*0746 and B\*5606 and 5614 alleles. Specific PCR fragment of 150 bp in the Cw\*0730 allele.

<sup>15</sup> Primer mix 26: Specific PCR fragment of 100 bp in the Cw\*0732N allele. Specific PCR fragment of 300 bp in the Cw\*0731 allele.

<sup>16</sup> Primer mix 27: Specific PCR fragment of 120 bp in the Cw\*0737 allele. Specific PCR fragment of 290 bp in the Cw\*070107, 0714 and 0734 alleles.

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

<b>INTERPRETATION TABLE</b>																
<b>HLA-Cw*07 SSP subtyping</b>																
<b>Amplification patterns of the Cw*0701 to Cw*0749 alleles</b>																
	<b>Tube<sup>4</sup></b>															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<b>Length of spec.</b>	80	290	110	185	245	100	190	285	240	185	275	130	80	240	290	395
<b>PCR product</b>								645	425		305					
<b>Length of int.</b>	<b>800</b>	<b>800</b>	<b>800</b>	1070	<b>800</b>	1070	1070	1070	<b>800</b>	1070	<b>800</b>	1070	1070	<b>800</b>	<b>800</b>	<b>800</b>
<b>pos. control<sup>1</sup></b>																
<b>5'-primer(s)<sup>2</sup></b>	1049	368	270	368	47	540	363	355	47	368	355	512	1049	419	369	47
	5'-Agg <sup>3'</sup>	5'-gTA <sup>3'</sup>	5'-AAC <sup>3'</sup>	5'-gTC <sup>3'</sup>	5'-Agg <sup>3'</sup>	5'-gAC <sup>3'</sup>	5'-AAT <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-Agg <sup>3'</sup>	5'-gTT <sup>3'</sup>	5'-TCA <sup>3'</sup>	5'-gTg <sup>3'</sup>	5'-Agg <sup>3'</sup>	5'-gTT <sup>3'</sup>	5'-TAC <sup>3'</sup>	5'-Agg <sup>3'</sup>
								992	419		385					
								5'-TAA <sup>3'</sup>	5'-gTA <sup>3'</sup>		5'-ggT <sup>3'</sup>					
<b>3'-primer(s)<sup>3</sup></b>	1087	618	341	512	121	601	512	601	302	512	618	601	1087	618	616	270
	5'-AgT <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-CgT <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-ggC <sup>3'</sup>	5'-CTT <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-CTT <sup>3'</sup>	5'-ggT <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-CTT <sup>3'</sup>	5'-AgC <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-TgT <sup>3'</sup>	5'-TAC <sup>3'</sup>
								1016	618							
								5'-CAC <sup>3'</sup>	5'-CCT <sup>3'</sup>							
<b>Tube No.</b>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<b>HLA-Cw allele</b>																
<b>*070101-070104, 070106</b>	+	+	+													
<b>*070105</b>	+		+													
<b>*070107</b>	+	+	+													
<b>*07020101-07020103</b>	+			+												+
<b>*0703</b>	+				+											+
<b>*070401-070402</b>	+					+										+
<b>*0705</b>	+	+					+									+
<b>*0706</b>	W	+	+					+								
<b>*0707</b>	?	+							+							
<b>*0708</b>	?									+						+
<b>*0709</b>	?	+	+						+							
<b>*0710</b>	?			+							+					+
<b>*0711</b>						+							+			+
<b>*0712</b>	?					+						+				+
<b>*0713</b>	?			+										+		+
<b>*0714</b>	?	+													+	+
<b>*0715</b>	?			+												+
<b>Tube No.</b>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

<b>INTERPRETATION TABLE</b>																
<b>HLA-Cw*07 SSP subtyping</b>																
<b>Amplification patterns of the Cw*0701 to Cw*0749 alleles</b>																
<b>Tube<sup>4</sup></b>																
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
130	230	220	145	225	250	155	130	105	100	120	260	255	275	415	245	<b>Length of spec. PCR product</b>
		465	170			185		150	300	290						<b>Length of int. pos. control<sup>1</sup></b>
1070	800	800	1070	800	800	1070	1070	1070	1070	1070	800	1070	1070	1070	1070	<b>5'-primer(s)<sup>2</sup></b>
512	368	47	486	6 <sup>th</sup> I	134	199	524	206	361	369	390	122	368	47	97	
5'-gTT <sup>3'</sup>	5'-gTC <sup>3'</sup>	5'-Agg <sup>3'</sup>	5'-TAC <sup>3'</sup>	5'-gTC <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-gTT <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-AgA <sup>3'</sup>	5'-AgT <sup>3'</sup>	5'-TAC <sup>3'</sup>	5'-gAA <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-gTC <sup>3'</sup>	5'-Agg <sup>3'</sup>	5'-TCg <sup>3'</sup>	
			490			504	539	419	ins 560	539	410	410				
			5'-CgA <sup>3'</sup>			5'-CAC <sup>3'</sup>	5'-gTg <sup>3'</sup>	5'-gTC <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-gCg <sup>3'</sup>	5'-gTg <sup>3'</sup>	5'-gTT <sup>3'</sup>				
			512													
			5'-gTg <sup>3'</sup>													
601	559	90	618	1043	341	341	618	270	618	618	618	341	603	289	302	<b>3'-primer(s)<sup>3</sup></b>
5'-CTT <sup>3'</sup>	5'-CTC <sup>3'</sup>	5'-Ag <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-CAA <sup>3'</sup>	5'-CgT <sup>3'</sup>	5'-CgT <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-TAC <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-CgT <sup>3'</sup>	5'-TTg <sup>3'</sup>	5'-AgT <sup>3'</sup>	5'-ggT <sup>3'</sup>
		97				618		527				618				
		5'-gTA <sup>3'</sup>				5'-CCT <sup>3'</sup>		5'-CCC <sup>3'</sup>				5'-CCT <sup>3'</sup>				
		341														
		5'-Cgg <sup>3'</sup>														
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	<b>Tube No.</b>
																<b>HLA-Cw allele</b>
																<b>*070101-070104, 070106</b>
																<b>*070105</b>
										+						<b>*070107</b>
																<b>*07020101-07020103</b>
			+													<b>*0703</b>
+																<b>*070401-070402</b>
																<b>*0705</b>
				+												<b>*0706</b>
		+													+	<b>*0707</b>
																<b>*0708</b>
															+	<b>*0709</b>
																<b>*0710</b>
+																<b>*0711</b>
																<b>*0712</b>
																<b>*0713</b>
										+						<b>*0714</b>
	+															<b>*0715</b>
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	<b>Tube No.</b>

Length of spec.	80	290	110	185	245	100	190	285	240	185	275	130	80	240	290	395
PCR product								645	425		305					
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*0716	?	+														
*0717	?															+
*0718	W	+	+													
*0719	W		+	+												
*0720	?	+	+													
*0721	?	+	+													
*0722	?	+	+													
*0723	?			+												+
*0724	?	+	+								+					
*0725	?			+												+
*0726	+	+	+													
*0727	?	+														+
*0728	?	+	+											+		
*0729	?			+					+							+
*0730	+	+	+													
*0731	?	+														+
*0732N	+			+												+
*0733N	?			+												+
*0734	?	+														+
*0735	?	+	+													
*0736	+	+	+													
*0737	?			+												+
*0738	?			+												+
*0739	?			+												+
*0740	?	+	+													
*0741	?											+				+
*0742	+			+												+
*0743	?	+									+					+
*0744	?	+	+													
*0745	?							+								+
*0746	?			+												+
*0747	?			+												+
*0748	?			+												+
*0749	+			+					+							+
*0214, 06020101- 060202, 0604-0616N																
*0315, 0332, 0403, 0406, 0416																
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16



HLA-Cw\*07  
 101.615-24/04 – licensed for PCR  
 101.615-24u/04u – not licensed for PCR  
 Lot No.: **X93**

130	230	220	145	225	250	155	130	105	100	120	260	255	275	415	245	Length of spec. PCR product
		465	170			185		150	300	290						Tube No.
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
		+														*0716
			+													*0717
				+												*0718
				+												*0719
					+											*0720
						+										*0721
							+									*0722
						+										*0723
							+									*0724
		+														*0725
																*0726
																*0727
																*0728
																*0729
								+								*0730
									+							*0731
									+							*0732N
		+														*0733N
										+						*0734
												+				*0735
												+				*0736
										+						*0737
													+			*0738
														+		*0739
														+		*0740
																*0741
												+				*0742
																*0743
			+													*0744
																*0745
								+								*0746
											+					*0747
			+													*0748
															+	*0749
															+	*0214, 06020101- 060202, 0604-0616N
					+											*0315, 0332, 0403, 0406, 0416
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.



Length of spec.	80	290	110	185	245	100	190	285	240	185	275	130	80	240	290	395
PCR product								645	425		305					
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*050101-050103, 0503-0508, 0510- 0516, 080101-0806, 0808-0814												+				
*0517		+												+		
*0807																
*1801-1803									+							+
HLA-Cw allele																
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A*0284								+								
A*2910												+				
B*0806, 0817																
B*2736							+									
B*4060				+												
B*5608, 5614																
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

<sup>1</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most tubes, or a band of 800 base pairs, for some tubes. Tube number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-C\*07 subtyping. In addition, tubes number 2, 3, 5, 9, 11, 14, 15, 16, 18, 19, 21, 22 and 28 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.

<sup>2</sup>The nucleotide position, in the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 5<sup>th</sup> or 7<sup>th</sup> exon or 6<sup>th</sup> 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.

<sup>3</sup>The nucleotide position, in 2<sup>nd</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 6<sup>th</sup> or 7<sup>th</sup> 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.

130	230	220	145	225	250	155	130	105	100	120	260	255	275	415	245	Length of spec. PCR product
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.
		465	170			185		150	300	290						*050101-050103, 0503-0508, 0510- 0516, 080101-0806, 0808-0814
			+													*0517
+																*0807
															+	*1801-1803
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	HLA-Cw allele
																Tube No.
																A*0284
																A*2910
															+	B*0806, 0817
																B*2736
	+												+			B*4060
								w								B*5608, 5614
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.

<sup>4</sup>Primer mix 8: Specific PCR fragment of 285 bp in the Cw\*0745 and A\*0284 alleles. Specific PCR fragment of 645 bp in the Cw\*0706 allele.

Primer mix 9: Specific PCR fragment of 240 bp in the Cw\*0729 allele. Specific PCR fragment of 425 bp in the Cw\*0707, 0709 and 0749 and the Cw\*1801-1802 alleles.

Primer mix 11: Specific PCR fragment of 275 bp in the Cw\*0724 allele. Specific PCR fragment of 305 bp in the Cw\*0710 and 0743 alleles.

Primer mix 19: Specific PCR fragment of 220 bp in the Cw\*0726 and 0733N alleles. Specific PCR fragment of 465 bp in the Cw\*0707 and 0716 alleles.

Primer mix 20: Specific PCR fragment of 145 bp in the Cw\*0703 and 0717 and Cw\*0517 alleles. Specific PCR fragment of 170 bp in the Cw\*0744 and 0748 alleles.

Primer mix 23: Specific PCR fragment of 155 bp in the Cw\*0723 allele. Specific PCR fragment of 185 bp in the Cw\*0721 allele.

Primer mix 25: Specific PCR fragment of 105 bp in the Cw\*0746 and B\*5606 and 5614 alleles. Specific PCR fragment of 150 bp in the Cw\*0730 allele.

Primer mix 26: Specific PCR fragment of 100 bp in the Cw\*0732N allele. Specific PCR fragment of 300 bp in the Cw\*0731 allele.

Primer mix 27: Specific PCR fragment of 120 bp in the Cw\*0737 allele. Specific PCR fragment of 290 bp in the Cw\*070107, 0714 and 0734 alleles.

'w', might be weakly amplified.

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

CELL LINE VALIDATION SHEET																				
HLA-Cw*07 SSP subtyping kit																				
				Tube																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Prod. No.:	200620201	200735102	200620203	200620204	200620205	200735106	200620207	200735108	200620209	200620210	200620211	200620212	200620213	200620214	200735115	200620216
	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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CELL LINE VALIDATION SHEET																			
HLA-Cw*07 SSP subtyping kit																			
				Tube															
				17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
				Prod. No.:															
				200735117	200620218	200620219	200735120	200620221	200735122	200735123	200620224	200735125	200620226	200620227	200735128	200735129	200620230	200735131	200735132
	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\*07 SSP

**Product number:** 101.615-24/04 – licensed for PCR  
101.615-24u/04u – not licensed for PCR  
**Lot number:** X93  
**Expiry date:** 2009-June-01  
**Number of tests:** 24 tests – Product No. 101.615-24/24u  
4 tests – Product No. 101.615-04/04u  
**Number of tubes per test:** 32

#### Tube specifications:

Tube No.	Production No.	Tube No.	Production No.	Tube No.	Production No.
1	2006-202-01	13	2006-202-13	25	2007-351-25
2	2007-351-02	14	2006-202-14	26	2006-202-26
3	2006-202-03	15	2007-351-15	27	2006-202-27
4	2006-202-04	16	2006-202-16	28	2007-351-28
5	2006-202-05	17	2007-351-17	29	2007-351-29
6	2007-351-06	18	2006-202-18	30	2006-202-30
7	2006-202-07	19	2006-202-19	31	2007-351-31
8	2007-351-08	20	2007-351-20	32	2007-351-32
9	2006-202-09	21	2006-202-21		
10	2006-202-10	22	2007-351-22		
11	2006-202-11	23	2007-351-23		
12	2006-202-12	24	2006-202-24		

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 5, 8, 10, 11, 13 to 15, 18 to 20, 23 to 31 were available. The specificities of the primers in primer solutions 10, 11, 13, 14, 18, 19, 20, 24, 26, 27, 30 and 31 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 5, 15 and 25 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 8, 23, 28 and 29 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-August-22

**Approved by:**

**Quality Control, Supervisor**

## Declaration of Conformity

**Product name:** Olerup SSP™ HLA-Cw\*07  
**Product number:** 101.615-24/04, 101.615-24u/04u  
**Lot number:** X93

**Intended use:** HLA-Cw\*07 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-August-22

Olle Olerup  
Managing Director

HLA-Cw\*07  
101.615-24/04 – licensed for PCR  
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Lot No.: **X93**

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101.615-24u/04u – not licensed for PCR  
Lot No.: **X93**

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101.615-24u/04u – not licensed for PCR  
Lot No.: **X93**

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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* SSP™ is a trademark of *Olerup* SSP AB.  
PCR™ is a trademark of F. Hoffmann-La Roche Ltd.  
ARMS™ is a trademark of Zeneca Ltd.

HLA-Cw\*07  
101.615-24/04 – licensed for PCR  
101.615-24u/04u – not licensed for PCR  
Lot No.: **X93**

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[www.olerup.com](http://www.olerup.com)

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

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