

HLA-B\*58  
101.568-06 – licensed for PCR  
101.568-06u – not licensed for PCR  
Lot No.: **Y35**

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## **Olerup SSP™ HLA-B\*58**

**Product number:** 101.568-06 – licensed for PCR  
101.568-06u - not licensed for PCR  
**Lot number:** Y35  
**Expiry date:** 2009-September-01  
**Number of tests:** 6  
**Number of tubes per test:** 16  
**Storage - pre-aliquoted primers:** dark at -20°C  
**- PCR Master Mix:** -20°C

**This Product Description is only valid for Lot No. Y35.**

### **CHANGES COMPARED TO THE PREVIOUS OLERUP SSP™ HLA-B\*58 LOT**

The HLA-B\*58 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP™* HLA-B\*58 lot was made (**Lot No. V83**).

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

<b>Tube</b>	<b>5'-primer</b>	<b>3'-primer</b>	<b>rationale</b>
4	-	Added	Primer added for the B*5815 allele.
9	Added	Added	Primer pair added for the B*5814 allele.

## PRODUCT DESCRIPTION

### HLA-B\*58 SSP subtyping

#### CONTENT

The primer set contains 5'- and 3'-primers for identifying the B\*5801 to B\*5815 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.

16 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 14 PCR reactions in a 16 well cut PCR plate. Wells 15 and 16 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16

The 16 well cut PCR plate is marked with 'B\*58 Y35'.

Well No. 1 is marked with '1'.

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

**Please note:** When removing each 16 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-B\*58 SSP subtypings will only be influenced by many other HLA-B alleles as primer mixes 2, 3, 6, 7, 9 to 11, 13 and 14 amplify many non-HLA-B\*58 alleles. In addition, the Cw\*0317 allele will be amplified by primer mix 3.

### UNIQUELY IDENTIFIED ALLELES

All the HLA-B\*58 alleles, i.e. **B\*5801 to B\*5815**, recognized by the HLA Nomenclature Committee in October 2007<sup>1</sup> will give rise to unique amplification patterns by the primers in the HLA-B\*58 subtyping kit.

<sup>1</sup>**Nomenclature for factors of the HLA system, 1998.** *Tissue Antigens* 1999; **53**: 407-446.  
HLA-B alleles listed on the IMGT/HLA web page 2007-October-05, release 2.19.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

### RESOLUTION IN HOMO- AND HETEROZYGOTES

The fourteen HLA-B\*58 alleles can be combined in 105 homozygous and heterozygous combinations. Forty-two of these genotypes do not give rise to unique amplification patterns.

+++++--+	-----+---	5804, 5806 = 5806, 5815
+++++---+	---+-----	5804, 5807 = 5807, 5815
+++++-----	---+-----	5802, 5804 = 5802, 5815
++-++----	-----+---	5804, 5805 = 5805, 5815
++-++-+-	+-----+---	5804, 5808 = 5808, 5815
++-+-----	+-----+---	5804, 5814 = 5814, 5815
++-+-----	-+-----+---	5804, 5809 = 5809, 5815
++-+-----	-----+---	5804, 5810N = 5810N, 5815
++-+-----	-----+---	5804, 5811 = 5811, 5815
++-+-----	-----+--+	5804, 5812 = 5812, 5815
++-+-----	-----+--+	5804, 5813 = 5813, 5815
++-+-----	-----+---	5801, 5804 = 5801, 5815 = 5804, 5815 = 5815, 5815
++-+-----	-----+---	5801, 5805 = 5805, 5805
++-+-----	+-----+---	5801, 5808 = 5808, 5814
++-+-----	+-----+---	5801, 5814 = 5814, 5814
++-+-----	-+-----+---	5801, 5809 = 5809, 5809
++-+-----	-----+---	5801, 5810N = 5810N, 5810N
++-+-----	-----+--+	5801, 5812 = 5812, 5812
++-+-----	-----+--+	5801, 5813 = 5813, 5813
++-+-----	---+-----	5802, 5807 = 5807, 5807

## LICENSES

### 101.568-212 – 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.

### 101.568-06u – 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.

### 101.568-06 and 101.568-06u

These products use ARMS™ technology and is sold under license from Zeneca Limited. ARMS is the subject of European Patent No. 0332435, US Patent No. 5595890 and corresponding world-wide patents. ARMS is a trademark of Zeneca Limited.

## GUARANTEE

Olerup SSP AB guarantees that the primers in the HLA-B\*58 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 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

#### **101.568-06 – licensed for PCR**

For one HLA-B\*58 subtyping, add at room temperature in a 0.5 ml tube:

18 x 2 μl = 36 μl DNA (30 ng/μl)

18 x 3 μl = 64 μl PCR Master Mix complete with *Taq* – mix well before taking your aliquot

18 x 5 μl = 90 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 16 wells of an HLA-B\*58 subtyping. **Well No. 1 of the 16 well PCR plate is marked with '1'**. Close the 16 well PCR plate with the provided lids.

#### **101.568-06u – not licensed for PCR**

For one HLA-B\*58 subtyping, add at room temperature in a 0.5 ml tube:

18 x 2 μl = 36 μl DNA (30 ng/μl)

18 x 3 μl = 64 μl PCR Master Mix without *Taq* – mix well before taking your aliquot

1.5 μl *Taq* polymerase (5 units/μl)

18 x 5 μl – 1.5 μl = 88.5 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 16 wells of an HLA-B\*58 subtyping. **Well No. 1 of the 16 well PCR plate is marked with '1'**. Close the 16 well PCR plate with the provided lids.

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

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**PCR cycling parameters:**

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

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

**AGAROSE GEL ELECTROPHORESIS**

Prepare a 2% (w/v) agarose gel in 0.5 x TBE buffer. Dissolve the agarose by boiling in a microwave oven. Let the gel solution cool to 60°C. Stain the gel prior to casting with ethidium bromide (10 mg/ml), 5 µl per 100 ml gel solution. For maximal ease of handling use our ethidium bromide dropper bottles (Product No. 103.301-10), 1 drop of ethidium bromide solution per 50-75 ml of gel. **Note: Ethidium bromide is a powerful carcinogen.**

Load the PCR products, preferably using an 8-channel pipette. Load a DNA size marker (100 base pair ladder, Product No. 103.201-100) in one well per row.

Run the gel in 0.5 x TBE buffer, without re-circulation of the buffer, for 15-20 minutes at 8-10 V/cm.

**DOCUMENTATION AND INTERPRETATION**

Put the gel on a UV transilluminator and document by photography.

Record the presence and absence of specific PCR products. The relative lengths of the specific PCR products are helpful in the interpretation of the results.

Record the presence and relative lengths of the internal positive control bands. The differently sized control bands will help in the correct orientation of the typing as well as in kit identification.

Lanes without either control band or specific PCR products should be repeated.

Interpret the typings with the ***lot-specific Interpretation and Specificity Tables***.

**INTERPRETATION SOFTWARE**

The interpretation software (Product No. 110.101) can be helpful in the interpretation of the typings.

## PCR MASTER MIXES

The PCR Master Mix complete with *Taq* contains:

<i>Taq</i> polymerase	0.4 unit per 10 $\mu$ 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-B\*58 SSP subtyping

Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-B\*58 SSP subtyping

Primer Mix	Approx. size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-B*58 alleles	Other amplified HLA Class I alleles <sup>3</sup>
<b>1<sup>4,6</sup></b>	90 bp	<b>800 bp</b>	5801, 5802, 5805-5815	
<b>2</b>	150 bp	<b>800 bp</b>	5801, 5804, 5805, 5809-5815	1301, 1306, 1307N, 1312, 1313, 1317, 1320, 1502, 1513, 1520, 1521, 1525, 1536, 1544, 1562, 1577, 1580, 1585, 1588, 1589, 9506, 9512, 9521, 9539, 1822, 2719, 2730, 350101-350402, 3506-350803, 3510-3517, 3519-3521, 3523-3530, 3533-3536, 3538-3542, 3545-3550, 3552-3557, 3559, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3582, 3583, 370101-370104, 3703N-3706, 3708, 3710, 3711, 3713, 3942, 4028, 44020101-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-4457, 4802, 5104, 5142, 5146, 530101-5306, 5308-5313, 5514, 5609, 5611, 5612, 570101-5710, 5712, 5714, 8301
<b>3</b>	240 bp	<b>800 bp</b>	5802, 5806, 5807	5711, <b>Cw*0317</b>
<b>4<sup>7</sup></b>	190, 230 bp	1070 bp	5804, 5815	
<b>5<sup>4</sup></b>	90 bp	1070 bp	5805	9524, 4077, 4814
<b>6</b>	170 bp	1070 bp	5806, 5808	070201-0724, 0726-0747, 0749N, 0750, 0752-0760, 0806, 0820, 1316, 1320, 1401-1407N, 15010101-150104, 150106-1504, 1506-1519, 1521, 1523-1530, 1532-1540, 1542-1547, 1549, 1550, 1553, 1554, 1556-1558, 1560-1574, 1576-1582, 1585, 1587, 1589, 1590, 1592-1599,



				9501-9504, 9506, 9508-9510, 9512, 9513, 9515-9522, 9525-9529, 9531-9535, 9537-9539, 1815, 1819, 1821, 270401-270402, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2725, 3511, 351401-351402, 3521, 3543, 3544, 3558, 3567, 3579, 3707, 3713, 3810, 3918, 3935, 3936, 4005, 4015, 4016, 4023, 4026, 4028, 4032, 4051, 460101-4605, 4607N, 4608, 4610, 4612, 4805, 4808, 4815, 4901-4905, 5001, 5002, 5004, 510101-5104, 5106, 5107, 5111N-5114, 5116-5118, 5121-5124, 5126-5130, 5132-5139, 5141N, 5143, 5145, 5146, 5148-5151, 520101-5202, 5204-5209, 5211, 5306, 5308, 5406, 550101-550104, 5503, 5505, 5509, 5511, 5514, 5515, 5517, 5521, 5524, 5525, 5528, 5603, 560501-5606, 5621, 5709, 5713, 7801-7803, 7805
<b>7<sup>4,5,6</sup></b>	80 bp	1070 bp	5806, 5808	1316, 1320, 1401-140202, 1403 <sup>weakly</sup> , 1404-1407N, 1542, 1815, 3511, 3521, 3558, 3810, 3935, 4028, 4901-4905, 5001, 5002, 5004, 510101-5104, 5106, 5107, 5111N-5114, 5116-5118, 5121-5124, 5126-5128, 5129 <sup>weakly</sup> , 5130, 5132-5135, 5137-5139, 5141N, 5143, 5145, 5146, 5148-5151, 520101-5202, 5204-5209, 5211, 5306, 5308, 550101-550104, 5503, 5505, 5509 <sup>weakly</sup> , 5511, 5514, 5515, 5517, 5524, 5525, 5528, 560501-5606, 5621, 7801-7803, 7805
<b>8</b>	250 bp	1070 bp	5807	
<b>9<sup>5</sup></b>	160 bp	<b>800 bp</b>	5808, 5814	5514, 5714
<b>10</b>	270 bp	<b>800 bp</b>	5809	1822, 3521, 3524, 3704, 4028, 5104, 5146, 5302, 5306, 5714
<b>11</b>	205 bp	1070 bp	5802, 5807	5711
<b>12</b>	235 bp	1070 bp	5810N	
<b>13<sup>8</sup></b>	165 bp	1070 bp	5801, 5802, 5804-5810N,	1520, 350101-3530, 3532-3542, 3544, 3545, 3547-3556, 3558, 3559, 3561-3572, 3574-3578, 3582,

			5812-5815	3583, 4802, 510101-5124, 5126-5141N, 5143-5146, 5148-5151, 520101-520602, 5208-5211, 530101-5313, 5621, 7801-7805, 8103
14 <sup>8</sup>	165 bp	1070 bp	5811	070201-0757, 0759, 0760, 080101-0834, 1301-1304, 1306-1321, 1401-1407N, 15010101-150104, 150106-1519, 1521, 1523-1540, 1542-1558, 1560-1599, 9501, 9503-9529, 9531-9539, 180101-1815, 1817N-1827, 2701-270402, 270502-2721, 2723-2738, 3531, 3543, 3546, 3557, 3560, 3579, 370101-3713, 380101-3817, 39010101-39010102L, 390103-3920, 3922-3943, 400101-4016, 4018-4040, 4042-4078, 4101-4108, 4201, 4202, 4204-4209, 44020101-4457, 4501-4507, 460101-4612, 47010101-4705, 480101-480102, 480301-4819, 4901-4905, 5001, 5002, 5004, 5207, 5401-5413, 550101-5505, 5507-5528, 5601-5620, 570101-5714, 5901, 5902, 670101-6702, 8101, 8102, 8201, 8202, 8301
15	155 bp	1070 bp	5812	
16	145 bp	1070 bp	5813	

<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-B\*58 SSP subtypings. 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.

<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-B\*58 subtyping. In addition, tubes number 2, 3, 9 and 10 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>Due to the sharing of sequence motifs between HLA-B alleles some non-HLA-B\*58 alleles will be amplified by primer mixes 2, 3, 6, 7, 9, 10, 11, 13 and 14. In addition, the Cw\*0317 allele will be amplified by primer mix 3.

<sup>4</sup>Short specific PCR fragments are less intense and not as sharp as longer specific bands.

<sup>5</sup>Primer mixes 7 and 9 has a tendency of giving rise to nonspecific amplifications.

<sup>6</sup>Primer mixes 1 and 7 have a tendency of primer dimer formation.

<sup>7</sup>Primer mix 4: Specific PCR fragment of 190 bp in the B\*5804 allele. Specific PCR fragment of 230 bp in the B\*5815 allele.

<sup>8</sup>Primer mixes 13 and 14: For many HLA-B alleles fourth exon nucleotide sequences are not available. We assume that unknown sequences in the fourth exon are conserved within allelic groups.

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

INTERPRETATION TABLE								
HLA-B*58 SSP subtyping								
Amplification patterns of the B*5801 to B*5815 alleles								
	Tube <sup>5</sup>							
	1	2	3	4	5	6	7	8
Length of spec.	90	150	240	190	90	170	80	250
PCR product				230				
Length of int.	800	800	800	1070	1070	1070	1070	1070
pos. control <sup>1</sup>								
5'-primer(s) <sup>2</sup>	209	355	361	106	463	527	486	361
	5'-ggA <sup>3'</sup>	5'-TCA <sup>3'</sup>	5'-AgT <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-TgA <sup>3'</sup>	5'-TgA <sup>3'</sup>	5'-ACC <sup>3'</sup>	5'-AgT <sup>3'</sup>
3'-primer(s) <sup>3</sup>	256	463	559	256	512	3 <sup>rd</sup> I	527	572
	5'-CCC <sup>3'</sup>	5'-gCT <sup>3'</sup>	5'-CAg <sup>3'</sup>	5'-CTC <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-TAT <sup>3'</sup>	5'-CCT <sup>3'</sup>	5'-gCg <sup>3'</sup>
				296				
				5'-CTg <sup>3'</sup>				
Tube No.	1	2	3	4	5	6	7	8
HLA-B allele <sup>4</sup>								
*5801	+	+						
*5802	+		+					
*5804		+		+				
*5805	+	+			+			
*5806	+		+			+	+	
*5807	+		+					+
*5808	+					+	+	
*5809	+	+						
*5810N	+	+						
*5811	+	+						
*5812	+	+						
*5813	+	+						
*5814	+	+						
*5815	+	+		+				
Tube No.	1	2	3	4	5	6	7	8

INTERPRETATION TABLE								
HLA-B*58 SSP subtyping								
Amplification patterns of the B*5801 to B*5815 alleles								
Tube <sup>5</sup>								
9	10	11	12	13	14	15	16	
160	270	205	235	165	165	155	145	Length of spec. PCR product
800	<b>800</b>	1070	1070	1070	1070	1070	1070	Length of int. pos. control <sup>1</sup>
141	355	361	366	652	652	142	454	5'-primer(s) <sup>2</sup>
5'-ATT <sup>3'</sup>	5'-TCA <sup>3'</sup>	5'-AgT <sup>3'</sup>	5'-ATT <sup>3'</sup>	5'-CCg <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-TCT <sup>3'</sup>	5'-ACA <sup>3'</sup>	
362								
5'-ggT <sup>3'</sup>								
256	585	527	559	774	774	256	559	3'-primer(s) <sup>3</sup>
5'-CCC <sup>3'</sup>	5'-gTg <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-CAG <sup>3'</sup>	5'-ggT <sup>3'</sup>	5'-ggT <sup>3'</sup>	5'-CCC <sup>3'</sup>	5'-CAG <sup>3'</sup>	
486								
5'-gCg <sup>3'</sup>								
9	10	11	12	13	14	15	16	Tube No. HLA-B allele <sup>4</sup>
				+				*5801
		+		+				*5802
				+				*5804
				+				*5805
				+				*5806
		+		+				*5807
+				+				*5808
	+			+				*5809
			+	+				*5810N
					+			*5811
				+		+		*5812
				+			+	*5813
+				+				*5814
				+				*5815
9	10	11	12	13	14	15	16	Tube No.

HLA-B\*58  
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Length of spec.	90	150	240	190	90	170	80	250
PCR product				230				
Tube No.	1	2	3	4	5	6	7	8
*070201-0724, 0726-0747, 0749N, 0750, 0752-0757, 0759, 0760, 0806, 0820, 15010101-150104, 150106, 1503, 1504, 1506-1512, 1514-1519, 1523, 1524, 1526N-1530, 1532-1535, 1537-1540, 1543, 1545-1547, 1549, 1550, 1553, 1554, 1556-1558, 1560, 1561, 1563-1574, 1576, 1578, 1579N, 1581, 1582, 1587, 1590, 1592-1599, 9501, 9503, 9504, 9508-9510, 9513, 9515-9520, 9522, 9525-9529, 9531-9535, 9537, 9538, 1819, 1821, 270401-270402, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2725, 3543, 3579, 3707, 3918, 3936, 4005, 4015, 4016, 4023, 4026, 4032, 4051, 460101-4605, 4607N, 4608, 4610, 4612, 4805, 4808, 4815, 5406, 5521, 5603, 5713						+		
Tube No.	1	2	3	4	5	6	7	8

HLA-B\*58  
 101.568-06 – licensed for PCR  
 101.568-06u – not licensed for PCR  
 Lot No.: **Y35**

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155	270	205	235	165	165	155	145	Length of spec. PCR product
9	10	11	12	13	14	15	16	Tube No.
					+			*070201-0724, 0726-0747, 0749N, 0750, 0752-0757, 0759, 0760, 0806, 0820, 15010101-150104, 150106, 1503, 1504, 1506-1512, 1514-1519, 1523, 1524, 1526N, 1530, 1532-1535, 1537-1540, 1543, 1545- 1547, 1549, 1550, 1553, 1554, 1556-1558, 1560, 1561, 1563-1574, 1576, 1578, 1579N, 1581, 1582, 1587, 1590, 1592- 1599, 9501, 9503, 9504, 9508-9510, 9513, 9515-9520, 9522, 9525-9529, 9531-9535, 9537, 9538, 1819, 1821, 270401-270402, 2706, 2710, 2715, 2718, 2720, 2721, 2724, 2725, 3543, 3579, 3707, 3918, 3936, 4005, 4015, 4016, 4023, 4026, 4032, 4051, 460101-4605, 4607N, 4608, 4610, 4612, 4805, 4808, 4815, 5406, 5521, 5603, 5713
9	10	11	12	13	14	15	16	Tube No.

Length of spec.	90	150	240	190	90	170	80	250
PCR product				230				
Tube No.	1	2	3	4	5	6	7	8
*0725, 0748, 0751, 080101-0805, 0807-0819N, 0821-0834, 130201-1304, 1308Q-1311, 1314, 1315, 1318, 1319, 1321, 1505, 1531, 1548, 1551, 1552, 1555, 1575, 1583, 1584, 1586, 1591, 9505, 9507, 9511N, 9514, 9523, 9536, 180101-1814, 1817N, 1818, 1820, 1823N-1827, 2701-2703, 270502-270510, 2707-2709, 2711-2714, 2716, 2717, 2723, 2726-2729, 2731-2738, 3531, 3560, 3702, 3709, 3712, 380101-3809, 3811-3817, 39010101-39010102L, 390103-3917, 3919, 3920, 3922-3934, 3937-3941, 3943, 400101-4004, 40060101-401403, 4018-4022N, 4024, 4025, 4027, 4029-4031, 4033-4040, 4042-4050, 4052-4076, 4078, 4101-4108, 4201, 4202, 4204-4209, 4415, 4418, 4420, 4431, 4441, 4447, 4453, 4454, 4501-4507, 4606, 4609, 4611, 47010101-4705, 480101-480102, 480301-4804, 4806, 4807, 4809-4813, 4816-4819, 5401-5405N, 5407-5413, 550201-550202, 5504, 5507, 5508, 5510, 5512, 5513, 5516, 5518-5520, 5522, 5523, 5526, 5527, 5601, 5602, 5604, 5607, 5608, 5610, 5613-5620, 5901, 5902, 670101-6702, 8101, 8102, 8201, 8202								
*0758, 9502						+		
*1301, 1306, 1307N, 1312, 1313, 1317, 1588, 2719, 2730, 3546, 3557, 370101-370104, 3703N, 3705, 3706, 3708, 3710, 3711, 3942, 44020101-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-4457, 5609, 5611, 5612, 570101-5708, 5710, 5712, 8301		+						
*1316, 1401-140202, 1404-1407N, 1542, 1815, 3810, 3935, 4901-4905, 5001, 5002, 5004, 5207, 550101-550104, 5503, 5505, 5511, 5515, 5517, 5524, 5525, 5528, 560501-5606						+	+	
Tube No.	1	2	3	4	5	6	7	8



155	270	205	235	165	165	155	145	Length of spec. PCR product Tube No.
9	10	11	12	13	14	15	16	
					+			*0725, 0748, 0751, 080101-0805, 0807-0819N, 0821-0834, 130201-1304, 1308Q-1311, 1314, 1315, 1318, 1319, 1321, 1505, 1531, 1548, 1551, 1552, 1555, 1575, 1583, 1584, 1586, 1591, 9505, 9507, 9511N, 9514, 9523, 9536, 180101-1814, 1817N, 1818, 1820, 1823N-1827, 2701-2703, 270502-270510, 2707-2709, 2711-2714, 2716, 2717, 2723, 2726-2729, 2731-2738, 3531, 3560, 3702, 3709, 3712, 380101-3809, 3811-3817, 39010101-39010102L, 390103-3917, 3919, 3920, 3922-3934, 3937-3941, 3943, 400101-4004, 40060101-401403, 4018-4022N, 4024, 4025, 4027, 4029-4031, 4033-4040, 4042-4050, 4052-4076, 4078, 4101-4108, 4201, 4202, 4204-4209, 4415, 4418, 4420, 4431, 4441, 4447, 4453, 4454, 4501-4507, 4606, 4609, 4611, 47010101-4705, 480101-480102, 480301-4804, 4806, 4807, 4809-4813, 4816-4819, 5401-5405N, 5407-5413, 550201-550202, 5504, 5507, 5508, 5510, 5512, 5513, 5516, 5518-5520, 5522, 5523, 5526, 5527, 5601, 5602, 5604, 5607, 5608, 5610, 5613-5620, 5901, 5902, 670101-6702, 8101, 8102, 8201, 8202
								*0758, 9502
					+			*1301, 1306, 1307N, 1312, 1313, 1317, 1588, 2719, 2730, 3546, 3557, 370101-370104, 3703N, 3705, 3706, 3708, 3710, 3711, 3942, 44020101-4414, 4416, 4417, 4419N, 4421-4430, 4432-4440, 4442-4446, 4448-4452N, 4455-4457, 5609, 5611, 5612, 570101-5708, 5710, 5712, 8301
					+			*1316, 1401-140202, 1404-1407N, 1542, 1815, 3810, 3935, 4901-4905, 5001, 5002, 5004, 5207, 550101-550104, 5503, 5505, 5511, 5515, 5517, 5524, 5525, 5528, 560501-5606
9	10	11	12	13	14	15	16	Tube No.

Length of spec.	90	150	240	190	90	170	80	250
PCR product				230				
Tube No.	1	2	3	4	5	6	7	8
*1320		+				+	+	
*1403, 5509						+	W	
*1502, 1513, 1521, 1525, 1536, 1544, 1562, 1577, 1580, 1585, 1589, 9506, 9512, 9521, 9539, 3713, 5709		+				+		
*1520, 350101-350402, 3506-350803, 3510, 3512, 3513, 3515-3517, 3519- 352002, 3523, 3525-3530, 3533-3536, 3538-3542, 3545, 3547-3550, 3552-3556, 3559, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3582, 3583, 4802, 530101-530104, 5303-5305, 5309-5313		+						
*9524, 4077, 4814					+			
*1822, 3704		+						
*3505, 350901-350902, 3518, 3522, 3532, 3537, 3551, 3564, 3566, 3568, 3572, 3575, 5105, 5108-5110, 5115, 5119, 5120, 5131, 5140, 5144N, 5203, 5210, 5307, 7804, 8103								
*3511, 5308		+				+	+	
*351401-351402		+				+		
*3521, 5104, 5146, 5306		+				+	+	
*3524, 5302		+						
*3544, 3567, 5136						+		
*3558, 510101-5103, 5106, 5107, 5111N- 5114, 5116-5118, 5121-5124, 5126-5128, 5130, 5132-5135, 5137-5139, 5141N, 5143, 5145, 5148-5151, 520101-5202, 5204-520602, 5208, 5209, 5211, 5621, 7801-7803, 7805						+	+	
*4028		+				+	+	
*5129						+	W	
*5142		+						
*5514		+				+	+	
*5711			+					
*5714		+						
HLA-B allele <sup>4</sup>								
Tube No.	1	2	3	4	5	6	7	8
Cw*0317			+					
Tube No.	1	2	3	4	5	6	7	8

155	270	205	235	165	165	155	145	Length of spec. PCR product Tube No.
9	10	11	12	13	14	15	16	Tube No.
					+			*1320
					+			*1403, 5509
					+			*1502, 1513, 1521, 1525, 1536, 1544, 1562, 1577, 1580, 1585, 1589, 9506, 9512, 9521, 9539, 3713, 5709
				+				*1520, 350101-350402, 3506-350803, 3510, 3512, 3513, 3515-3517, 3519- 352002, 3523, 3525-3530, 3533-3536, 3538-3542, 3545, 3547-3550, 3552-3556, 3559, 3561-3563, 3565Q, 3569-3571, 3574, 3576-3578, 3582, 3583, 4802, 530101-530104, 5303-5305, 5309-5313
					+			*9524, 4077, 4814
	+				+			*1822, 3704
				+				*3505, 350901-350902, 3518, 3522, 3532, 3537, 3551, 3564, 3566, 3568, 3572, 3575, 5105, 5108-5110, 5115, 5119, 5120, 5131, 5140, 5144N, 5203, 5210, 5307, 7804, 8103
				+				*3511, 5308
				+				*351401-351402
	+			+				*3521, 5104, 5146, 5306
	+			+				*3524, 5302
				+				*3544, 3567, 5136
				+				*3558, 510101-5103, 5106, 5107, 5111N- 5114, 5116-5118, 5121-5124, 5126-5128, 5130, 5132-5135, 5137-5139, 5141N, 5143, 5145, 5148-5151, 520101-5202, 5204-520602, 5208, 5209, 5211, 5621, 7801-7803, 7805
	+				+			*4028
				+				*5129
								*5142
+					+			*5514
		+			+			*5711
+	+				+			*5714
HLA-B allele <sup>4</sup>								
9	10	11	12	13	14	15	16	Tube No.
Cw*0317								
9	10	11	12	13	14	15	16	Tube No.

<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-B\*58 subtyping.

In addition, tubes number 2, 3, 9 and 10 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 2<sup>nd</sup>, 3<sup>rd</sup> or 4<sup>th</sup> exon, 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 the 2<sup>nd</sup>, 3<sup>rd</sup> or 4<sup>th</sup> exon or the 3<sup>rd</sup> intron 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.

<sup>4</sup>The B\*5803 allele was never assigned.

<sup>5</sup>Primer mix 4: Specific PCR fragment of 190 bp in the B\*5804 allele. Specific PCR fragment of 230 bp in the B\*5815 allele.

'w', might be weakly amplified.

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

CELL LINE VALIDATION SHEET																					
HLA-B*58 SSP subtyping kit																					
				Prod. No.:	Tube																
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
					200618101	200618102	200618103	200738004	200618105	200618106	200618107	200618108	200738009	200618110	200618111	200618112	200618113	200618114	200624685	200624716	
	cell line		B*																		
1	9001 SA		*0702		-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
2	9280 LK707		*5201	*7301	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-
3	9011 E4181324		*52011		-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-
4	9275 GU373		*1510	*5301	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-
5	9009 KAS011		*3701		-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
6	9353 SM		*3901	*5101	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-
7	9020 QBL		*1801		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
8	9007 DEM		*5701		-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
9	9026 YAR		*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
10	9107 LKT3		*5401		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
11	9051 PITOUT		*440301		-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
12	9052 DBB		*5701		-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
13	9067 BTB		*270502		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
14	9071 OLGA		*1501	*1520	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-
15	9075 DKB		*4001		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
16	9037 SWEIG007		*4002		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
17	9008 WILJON		*1801		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
18	9257 32367		*1401	*5601	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-
19	9038 BM16		*1801		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
20	9059 SLE005		*4001		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
21	9064 AMALA		*1501		-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
22	9056 KOSE		*3503		-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
23	9124 IHL		*4002	*5602	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
24	9035 JBUSH		*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
25	9049 IBW9		*1402		-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-
26	9285 WT49		*5801		+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
27	9191 CH1007		*0705	*5101	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-
28	9320 BEL5GB		*4402	*4403	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
29	9050 MOU		*440301		-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
30	9021 RSH		*4201		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
31	9019 DUCAF		*1801		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
32	9297 HAG		*4102		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
33	9098 MT14B		*4001		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
34	9104 DHIF		*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
35	9302 SSTO		*4402		-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
36	9024 KT17		*1501	*3501	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-
37	9065 HHKB		*0702		-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
38	9099 LZL		*1501		-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
39	9315 CML		*0801	*2705	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
40	9134 WHONP199		*1302	*4601	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
41	9055 H0301		*1402		-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-
42	9066 TAB089		*4601		-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
43	9076 T7526		*4601		-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
44	9057 TEM		*3801		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
45	9239 SHJO		*4201	*5001	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-
46	9013 SCHU		*0702		-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
47	9045 TUBO		*5101		-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-
48	9303 TER-ND		*3501	*4403	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
49	9366 DAUDI		*5801	*5802	+	+	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-

## CERTIFICATE OF ANALYSIS

### Olerup SSP™ HLA-B\*58 SSP

Product number: 101.568-06 – licensed for PCR  
101.568-06u – not licensed for PCR  
Lot number: Y35  
Expiry date: 2009-September-01  
Number of tests: 6  
Number of tubes per test: 16

#### Tube specifications:

Tube No.	Production No.	Tube No.	Production No.
1	2006-181-01	9	2007-380-09
2	2006-181-02	10	2006-181-10
3	2006-181-03	11	2006-181-11
4	2007-380-04	12	2006-181-12
5	2006-181-05	13	2006-181-13
6	2006-181-06	14	2006-181-14
7	2006-181-07	15	2006-247-15
8	2006-181-08	16	2006-247-16

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

No DNAs carrying the alleles to be amplified by primer solutions 4, 5, 8, 9, 10, 12, 15 and 16 were available. The specificities of the primers in primer solutions 5, 8, 9, 10 and 15 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 4 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 12 and 16 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-December-21

**Approved by:**

**Quality Control, Supervisor**

## Declaration of Conformity

**Product name:** Olerup SSP™ HLA-B\*58  
**Product number:** 101.568-06, 101.568-06u  
**Lot number:** Y35

**Intended use:** HLA-B\*58 high resolution histocompatibility testing

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

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

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

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

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

Saltsjöbaden, Sweden  
2007-December-21

Olle Olerup  
Managing Director

HLA-B\*58  
101.568-06 – licensed for PCR  
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Lot No.: **Y35**

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HLA-B\*58  
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HLA-B\*58  
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Lot No.: **Y35**

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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.

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HLA-B\*58  
101.568-06 – licensed for PCR  
101.568-06u – not licensed for PCR  
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[www.olerup.com](http://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](mailto: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](mailto: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](mailto:info.us@olerup.com)

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

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