

Olerup SSP™ HLA-B*46

Product number: 101.544-06 – licensed for PCR
101.544-06u – not licensed for PCR
Lot number: Y24
Expiry date: 2009-September-01
Number of tests: 6
Number of tubes per test: 14
Storage - pre-aliquoted primers: dark at -20°C
- PCR Master Mix: -20°C

This Product Description is only valid for Lot No. Y24.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP™ HLA-B*46 LOT.

The HLA-B*46 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP™* HLA-B*46 lot (**Lot No. V67**).

Four tubes have been added to the HLA-B*46 kit,
wells **11 to 14**.

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

Tube	5'-primer	3'-primer	rationale
3	-	Modified	Increased specificity of specific primer pair.
8	-	Modified	Increased specificity of specific primer pair.
11	New	New	New primer pair for the B*4609 allele.
12	New	New	New primer pair for the B*4610 allele.
13	New	New	New primer pair for the B*4611 allele.
14	New	New	New primer pair for the B*4612 allele.

PRODUCT DESCRIPTION

HLA-B*46 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*4601 to B*4612 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.

14 PCR reactions with a reaction volume of 10 µl are performed per HLA-B*46 subtyping.

PLATE LAYOUT

Each HLA-B*46 test consists of 14 PCR reactions in a 16 well cut PCR plate. Wells 15 to 16 are empty.

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

The 16 well PCR plate is marked with 'B*46 Y24'.

Tube 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*46 SSP subtypings will be influenced by the many other HLA-B alleles. In addition, the A*3304 allele will be amplified by primer mix 8.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*46, i.e. **B*4601 to B*4612**, recognized by the HLA Nomenclature Committee in October 2007¹ will be amplified by the primers in the HLA-B*46 SSP kit.

The B*46 primer set cannot separate the B*460101 and B*460102 alleles.

¹**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.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 12 HLA-B*46 alleles can be combined in 78 homozygous and heterozygous combinations. Twelve of these genotypes do not give rise to unique amplification patterns.

++--+-	+-----	4601,4602 = 4602,4602
+---+--	+-----	4601,4607N = 4607N,4607N
+---+--	-----+	4608,4611 = 4611,4611
+---+--	+--+---	4601,4609 = 4609,4609
+---+--	+--+---	4601,4610 = 4610,4610
+---+--	+-----+	4601,4612 = 4612,4612

LICENSES

101.544-06 – 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.

101.544-06u – 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.

101.544-06 and 101.544-06u

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

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

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

The kit is shipped at ambient temperature.

PROTOCOL

DNA EXTRACTION

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

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

IMPORTANT:

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

DNA extracted by other methods, 30 ng/μl.

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

PCR AMPLIFICATION

101.544-06 – licensed for PCR

For one HLA-B*46 SSP subtyping, add at room temperature in a 0.5 ml tube:

16 x 2 μl = 32 μl DNA (30 ng/μl)

16 x 3 μl = 48 μl PCR Master Mix complete with *Taq* – mix well
before taking your aliquot

16 x 5 μl = 80 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-H₂O mixture into each of the 14 wells of an HLA-B*46 subtyping. **Well No. 1 of the 16 well PCR plate is marked with '1'**. Close the 16 well PCR plate with the provided lids.

101.544-06u – not licensed for PCR

For one HLA-B*46 SSP subtyping, add at room temperature in a 0.5 ml tube:

16 x 2 μl = 32 μl DNA (30 ng/μl)

16 x 3 μl = 48 μl PCR Master Mix without *Taq* – mix well before
taking your aliquot

1.3 μl *Taq* polymerase (5 units/μl)

16 x 5 μl – 1.3 μl = 78.7 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-*Taq*-H₂O mixture into each of the 14 wells of an HLA-B*46 subtyping. **Well No. 1 of the 16 well PCR plate is marked with '1'**. Close the 16 well PCR plate with the provided lids.

Lot No.: **Y24**

www.olerup.com

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

PCR cycling parameters:

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

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

AGAROSE GEL ELECTROPHORESIS

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

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

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

DOCUMENTATION AND INTERPRETATION

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

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

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

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

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

INTERPRETATION SOFTWARE

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

PCR MASTER MIXES

The PCR Master Mix complete with *Taq* contains:

<i>Taq</i> polymerase	0.4 unit per 10 μ l SSP reaction
nucleotides	final concentration of each dNTP is 200 μ M
PCR buffer	final concentrations: 50 mM KCl, 1.5 mM MgCl ₂ , 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin
glycerol	final concentration of glycerol is 5%
cresol red	final concentration of cresol red is 100 μ g/ml

The same PCR Master Mix complete with *Taq* is used for all the licensed *Olerup* SSP kits.

The PCR Master Mix without *Taq* contains:

nucleotides	final concentration of each dNTP is 200 μ M
PCR buffer	final concentrations: 50 mM KCl, 1.5 mM MgCl ₂ , 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin
glycerol	final concentration of glycerol is 5%
cresol red	final concentration of cresol red is 100 μ g/ml

The same PCR Master Mix without *Taq* is used for all the unlicensed *Olerup* SSP kits.

The PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* can be shipped at ambient temperature.

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

Vials with the PCR Master Mixes can be kept at $+4^{\circ}\text{C}$ for 4 weeks, but the PCR Master Mixes are then no longer stable for 24 months.

SPECIFICITY TABLE

HLA-B*46 SSP subtyping

Specificities and sizes of the PCR products of the 14 primer mixes used for HLA-B*46 SSP subtyping

Primer Mix	Approx. Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*46 alleles	Other amplified HLA Class I alleles ³
1⁴	130 bp	800 bp	460101-4603, 4605-4612	1557 ^{weakly}
2⁴	125 bp	1070 bp	4602	1318, 1403, 1547, 1549, 1565, 1584, 1599, 1813, 350801- 350803, 3545, 3561, 3566, 3908 ^{weakly} , 3911 ^{weakly} , 3933, 4404, 4428, 4456N, 5105, 5129, 5311, 5410, 5509, 5702, 5704, 5705, 5712
3⁴	115 bp	1070 bp	4603	
4⁴	130 bp	1070 bp	4604	0815, 1557 ^{weakly} , 3574, 4073, 5503
5	235 bp	1070 bp	460101-4604, 4606-4612	
6	230 bp	800 bp	4606	0709, 0711, 9538, 3566, 4038, 4052, 4059, 4209, 4814
7⁴	140 bp	1070 bp	4607N	1407N, 3940N, 5619N
8^{4,5}	115 bp	1070 bp	4608, 4611	1301-1304, 1306, 1307N, 1309-1317, 1319, 1320, 1401- 140202, 1404-1407N, 1502, 1503, 1505, 1506, 1509, 1510, 1513, 1516-1518, 1520, 1521, 1523, 1525, 1529, 1531, 1536, 1537, 1539, 1540, 1542, 1544, 1548, 1552, 1555, 1561, 1562, 1564, 1567, 1569, 1574, 1580, 1586, 1588, 1589, 1591, 1593, 1595, 1598, 9503, 9506, 9508, 9512, 9514, 9515, 9519, 9521, 9523, 9524, 9527, 9531-9534, 9536, 9538, 9539, 180101- 1812, 1814, 1815, 1817N, 1818, 1820, 1822-1827, 2701- 270402, 270502-2706, 2708-

				2710, 2712-2719, 2721, 2723, 2726-2732, 2735, 2737, 2738, 350101, 350103-3507, 3510- 3513, 3515-3517, 3519-3530, 3532-3537, 3539-3542, 3546- 3560, 3563, 3565Q, 3568- 3572, 3574-3578, 3582, 3583, 3702, 380101-3817, 39010101-39010102L, 390103-3920, 3922-3932, 3934, 3935, 3937-3943, 4026, 4028, 4035, 4071, 4077, 440301-440303, 4407, 4410, 4413, 4426, 4429, 4430, 4432, 4436-4440, 4443, 4445- 4447, 4450, 4454, 4457, 47010101-4705, 4802, 4812, 4814, 4901-4905, 5001, 5002, 5004, 510101-5104, 5106, 5107, 510901-510902, 5111N-5119, 5121-5123, 5126-5128, 5130-5135, 5137- 5143, 5145, 5146, 5148-5151, 520101-5211, 530101-5310, 5312, 5313, 5401-5405N, 5407-5409, 5412, 5413, 550101-5503, 5505, 5507, 5510-5519, 5522-5528, 5601, 5602, 5604-5612, 5614- 5619N, 5621, 570101- 570103, 570301-570302, 5706-5708, 5710, 5711, 5714, 5801, 5802, 5804-5815, 5901, 670101-6702, 7801-7805, A*3304 15010101-150104, 150106, 1504, 1507, 1508, 151101- 1512, 1514, 1515, 1519, 1524, 1526N-1528, 1530, 1532, 1534, 1535, 1538, 1543, 1545, 1546, 1550, 1553, 1554, 1556-1558, 1560, 1563, 1566, 1568, 1570, 1571, 1573, 1575-1577, 1579N, 1581, 1582, 1585, 1587, 1592, 1594N, 1596, 1597, 9501, 9502, 9504, 9505, 9509-9511N, 9513,
9⁴	115 bp	1070 bp	460101-4602, 4604, 4605, 4607N, 4609, 4610, 4612	

				9517, 9518, 9520, 9522, 9525, 9526, 9528, 9529, 9535, 9537, 1819, 2725, 351401-351402, 3543, 3544, 3562, 3567, 3579 ^{weakly} , 3918, 3936, 5406, 5521, 5603
10⁴	100 bp	800 bp	4605	9525, 3554
11	150 bp	1070 bp	4609	3562
12	170 bp	1070 bp	4610	
13⁴	105 bp	1070 bp	4611	130201-1303, 1308Q, 1309, 1314-1316, 1318, 1319, 1542, 3560, 4415, 4418, 4501, 4503- 4507, 4901-4903, 5001, 5002, 5004, 5115, 5401-5403, 5405N, 5407, 5408N, 5410, 5412, 5413, 550101-5503, 5505, 5507, 5509-5512, 5515, 5516, 5518, 5519, 5521, 5522, 5524-5526, 5601, 5607, 5608, 5613, 5614, 5616, 5617, 5619N, 5620, 5901
14	215 bp	1070 bp	4612	0755, 1507, 1545, 1568, 9526, 4819

¹Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of HLA-B*46 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.

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

Tube number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*46 SSP subtyping.

In addition, tubes number 6 and 10 contain the primer pair giving rise to the shorter, 800 bp, internal positive control in order to allow kit identification.

In the presence of a specific amplification the intensity of the control band often decreases.

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.

³Due to the sharing of sequence motifs between HLA-B alleles one non-HLA-B*46 alleles will be amplified by primer mixes 1, 3, 5, 7 to 11, 13 and 14 In addition, the A*3304 allele will be amplified by primer mix 8.

⁴Specific PCR fragments shorter than 150 base pairs are less intense and not as sharp as longer specific bands.

⁵Primer mix 8 may give rise to a primer oligomer artifact.

HLA-B*46
101.544-06 – licensed for PCR
101.544-06u – not licensed for PCR
Lot No.: **Y24**

11

www.olerup.com

INTERPRETATION TABLE								
HLA-B*46 SSP subtyping								
Amplification patterns of the B*4601 to 4612 alleles								
	Tube							
	1	2	3	4	5	6	7	8
Length of spec.	130	125	115	130	235	230	140	115
PCR product								
Length of int.	800	1070	1070	1070	1070	800	1070	1070
pos. control1								
5'-primer2	209	209	463	209	106	419	463	463
	5'-ggC ^{3'}	5'-ggC ^{3'}	5'-TgA ^{3'}	5'-ggg ^{3'}	5'-CCA ^{3'}	5'-gTC ^{3'}	5'-TgA ^{3'}	5'-TgA ^{3'}
3'-primer(s)3	299	293	538	299	299	610	564	539
	5'TCA ^{3'}	5'-ggC ^{3'}	5'-CCg ^{3'}	5'TCA ^{3'}	5'TCA ^{3'}	5'CTC ^{3'}	5'ACT ^{3'}	5'-TCA ^{3'}
								544
								5'-ggT ^{3'}
Tube No.	1	2	3	4	5	6	7	8
HLA-B allele								
*460101-460102	+				+			
*4602	+	+			+			
*4603	+		+		+			
*4604				+	+			
*4605	+							
*4606	+				+	+		
*4607N	+				+		+	
*4608	+				+			+
*4609	+				+			
*4610	+				+			
*4611	+				+			+
*4612	+				+			
*0709, 0711, 4038, 4052, 4059, 4209						+		
*0755, 4819								
*0815, 4073					+			
Tube No.	1	2	3	4	5	6	7	8

INTERPRETATION TABLE						
HLA-B*46 SSP subtyping						
Amplification patterns of the B*4601 to 4612 alleles						
Tube						
9	10	11	12	13	14	
115	100	150	170	105	215	Length of spec.
						PCR product
1070	800	1070	1070	1070	1070	Length of int.
						pos. control¹
463	106	419	142	357	363	5'-primer²
5'-TgA ^{3'}	5'-CCg ^{3'}	5'-gTC ^{3'}	5'-TCA ^{3'}	5'-Tgg ^{3'}	5'-AgC ^{3'}	
538	165	527	269	420	538	3'-primer(s)³
5'-CCA ^{3'}	5'-Tgg ^{3'}	5'-CCA ^{3'}	5'-ACT ^{3'}	5'-gCT ^{3'}	5'-CCA ^{3'}	
9	10	11	12	13	14	Tube No.
						HLA-B allele
+						*460101-460102
+						*4602
						*4603
+						*4604
+	+					*4605
						*4606
+						*4607N
						*4608
+		+				*4609
+			+			*4610
				+		*4611
+					+	*4612
						*0709, 0711, 4038, 4052, 4059, 4209
					+	*0755, 4819
						*0815, 4073
9	10	11	12	13	14	Tube No.

Length of spec.	130	125	115	130	235	230	140	115
PCR product								
Tube No.	1	2	3	4	5	6	7	8
*1301, 1304, 1306, 1307N, 1310-1313, 1317, 1320, 1401-140202, 1404-140602, 1502, 1503, 1505, 1506, 1509, 1510, 1513, 1516-1518, 1520, 1521, 1523, 1525, 1529, 1531, 1536, 1537, 1539, 1540, 1544, 1548, 1552, 1555, 1561, 1562, 1564, 1567, 1569, 1574, 1580, 1586, 1588, 1589, 1591, 1593, 1595, 1598, 9503, 9506, 9508, 9512, 9514, 9515, 9519, 9521, 9523, 9524, 9527, 9531-9534, 9536, 9539, 180101-1812, 1814, 1815, 1817N, 1818, 1820, 1822-1827, 2701-270402, 270502-2706, 2708-2710, 2712-2719, 2721, 2723, 2726-2732, 2735, 2737, 2738, 350101, 350103-3507, 3510-3513, 3515-3517, 3519-3530, 3532-3537, 3539-3542, 3546-3553N, 3555-3559, 3563, 3565Q, 3568-3572, 3575-3578, 3582, 3583, 3702								+
380101-3817, 39010101-39010102L, 390103-3907, 3909, 3910, 3912-3917, 3919, 3920, 3922-3932, 3934, 3935, 3937-3939, 3941-3943, 4026, 4028, 4035, 4071, 4077, 440301-440303, 4407, 4410, 4413, 4426, 4429, 4430, 4432, 4436-4440, 4443, 4445-4447, 4450, 4454, 4457, 47010101-4705, 4802, 4812, 4904, 4905, 510101-5104, 5106, 5107, 510901-510902, 5111N-5114, 5116-5119, 5121-5123, 5126-5128, 5130-5135, 5137-5143, 5145, 5146, 5148-5151, 520101-5211, 530101-5310, 5312, 5313, 5404, 5409, 5513, 5514, 5517, 5523, 5527, 5528, 5602, 5604-5606, 5609-5612, 5615, 5618, 5621, 570101-570103, 570301-570302, 5706-5708, 5710, 5711, 5714, 5801, 5802, 5804-5815, 670101-6702, 7801-7805								+
Tube No.	1	2	3	4	5	6	7	8

115	100	150	170	105	215	Length of spec. PCR product
9	10	11	12	13	14	Tube No.
						*1301, 1304, 1306, 1307N, 1310-1313, 1317, 1320, 1401-140202, 1404-140602, 1502, 1503, 1505, 1506, 1509, 1510, 1513, 1516-1518, 1520, 1521, 1523, 1525, 1529, 1531, 1536, 1537, 1539, 1540, 1544, 1548, 1552, 1555, 1561, 1562, 1564, 1567, 1569, 1574, 1580, 1586, 1588, 1589, 1591, 1593, 1595, 1598, 9503, 9506, 9508, 9512, 9514, 9515, 9519, 9521, 9523, 9524, 9527, 9531 9534, 9536, 9539, 180101-1812, 1814, 1815, 1817N, 1818, 1820, 1822-1827, 2701-270402, 270502-2706, 2708-2710, 2712-2719, 2721, 2723, 2726-2732, 2735, 2737, 2738, 350101, 350103-3507, 3510- 3513, 3515-3517, 3519-3530, 3532-3537, 3539-3542, 3546-3553N, 3555-3559, 3563, 3565Q, 3568-3572, 3575-3578, 3582, 3583, 3702
						380101-3817, 39010101-39010102L, 390103-3907, 3909, 3910, 3912-3917, 3919, 3920, 3922-3932, 3934, 3935, 3937- 3939, 3941-3943, 4026, 4028, 4035, 4071, 4077, 440301-440303, 4407, 4410, 4413, 4426, 4429, 4430, 4432, 4436-4440, 4443, 4445-4447, 4450, 4454, 4457, 47010101- 4705, 4802, 4812, 4904, 4905, 510101- 5104, 5106, 5107, 510901-510902, 5111N- 5114, 5116-5119, 5121-5123, 5126-5128, 5130-5135, 5137-5143, 5145, 5146, 5148- 5151, 520101-5211, 530101-5310, 5312, 5313, 5404, 5409, 5513, 5514, 5517, 5523, 5527, 5528, 5602, 5604-5606, 5609-5612, 5615, 5618, 5621, 570101-570103, 570301-570302, 5706-5708, 5710, 5711, 5714, 5801, 5802, 5804-5815, 670101- 6702, 7801-7805
9	10	11	12	13	14	Tube No.

Length of spec.	130	125	115	130	235	230	140	115
PCR product								
Tube No.	1	2	3	4	5	6	7	8
*130201-1303, 1309, 1314-1316, 1319, 1542, 3560, 4901-4903, 5001, 5002, 5004, 5115, 5401-5403, 5405N, 5407, 5408N, 5412, 5413, 550101-550202, 5505, 5507, 5510-5512, 5515, 5516, 5518, 5519, 5522, 5524-5526, 5601, 5607, 5608, 5614, 5616, 5617, 5901								+
*1308Q, 4415, 4418, 4501, 4503-4507, 5613, 5620								
*1318, 5410, 5509			+					
*1403, 1547, 1549, 1565, 1584, 1599, 1813, 350801-350803, 3545, 3561, 3933, 4404, 4428, 4456N, 5105, 5129, 5311, 5702, 5704, 5705, 5712			+					
*1407N, 3940N,							+	+
*15010101-150104, 150106, 1504, 1508, 151101-1512, 1514, 1515, 1519, 1524, 1526N-1528, 1530, 1532, 1534, 1535, 1538, 1543, 1546, 1550, 1553, 1554, 1556, 1558, 1560, 1563, 1566, 1570, 1571, 1573, 1575-1577, 1579N, 1581, 1582, 1585, 1587, 1592, 1594N, 1596, 1597, 9501, 9502, 9504, 9505, 9509-9511N, 9513, 9517, 9518, 9520, 9522, 9528, 9529, 9535, 9537, 1819, 2725, 351401-351402, 3543, 3544, 3567, 3936, 5406, 5603								
*1507, 1545, 1568, 9526								
*1557	W				W			
*9525								
*9538, 4814						+		+
*3554								+
*3562								
*3566			+			+		
*3574					+			+
*3579								
*3908, 3911			W					+
*3918								+
*5503					+			+
*5521								
Tube No.	1	2	3	4	5	6	7	8

115	100	150	170	105	215	Length of spec. PCR product
9	10	11	12	13	14	Tube No.
				+		*130201-1303, 1309, 1314-1316, 1319, 1542, 3560, 4901-4903, 5001, 5002, 5004, 5115, 5401-5403, 5405N, 5407, 5408N, 5412, 5413, 550101-550202, 5505, 5507, 5510-5512, 5515, 5516, 5518, 5519, 5522, 5524-5526, 5601, 5607, 5608, 5614, 5616, 5617, 5901
				+		*1308Q, 4415, 4418, 4501, 4503-4507, 5613, 5620
				+		*1318, 5410, 5509
						*1403, 1547, 1549, 1565, 1584, 1599, 1813, 350801-350803, 3545, 3561, 3933, 4404, 4428, 4456N, 5105, 5129, 5311, 5702, 5704, 5705, 5712
						*1407N, 3940N,
+						*15010101-150104, 150106, 1504, 1508, 151101-1512, 1514, 1515, 1519, 1524, 1526N-1528, 1530, 1532, 1534, 1535, 1538, 1543, 1546, 1550, 1553, 1554, 1556, 1558, 1560, 1563, 1566, 1570, 1571, 1573, 1575-1577, 1579N, 1581, 1582, 1585, 1587, 1592, 1594N, 1596, 1597, 9501, 9502, 9504, 9505, 9509-9511N, 9513, 9517, 9518, 9520, 9522, 9528, 9529, 9535, 9537, 1819, 2725, 351401-351402, 3543, 3544, 3567, 3936, 5406, 5603
+					+	*1507, 1545, 1568, 9526
+						*1557
+	+					*9525
						*9538, 4814
	+					*3554
+		+				*3562
						*3566
						*3574
W						*3579
						*3908, 3911
+						*3918
				+		*5503
+				+		*5521
9	10	11	12	13	14	Tube No.

Length of spec.	130	125	115	130	235	230	140	115
PCR product								
Tube No.	1	2	3	4	5	6	7	8
*5619N							+	+
HLA-B allele								
Tube No.	1	2	3	4	5	6	7	8
A*3304								+
Tube No.	1	2	3	4	5	6	7	8

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most tubes, or a band of 800 base pairs, for some tubes. Tube number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*46 SSP subtyping. In addition, tubes number 6 and 10 contain the primer pair giving rise to the shorter, 800 bp, internal positive control in order to allow kit identification.

115	100	150	170	105	215	Length of spec.
						PCR product
9	10	11	12	13	14	Tube No.
				+		*5619N
						HLA-B allele
9	10	11	12	13	14	Tube No.
						A*3304
9	10	11	12	13	14	Tube No.

²The nucleotide position, in the 2nd or 3rd 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.

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

'w', might be weakly amplified.

CELL LINE VALIDATION SHEET																			
HLA-B*46 SSP primer set																			
					Prod. No.:	Tube													
						1	2	3	4	5	6	7	8	9	10	11	12	13	14
						200623201	200623202	200737903	200623204	200623205	200623206	200623207	200737908	200623209	200623210	200737911	200737912	200737913	200737914
	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		*4403			-	-	-	-	-	-	-	+	-	-	-	-	-	-
12	9052 DBB		*5701			-	-	-	-	-	-	-	+	-	-	-	-	-	-
13	9067 BTB		*2705			-	-	-	-	-	-	-	+	-	-	-	-	-	-
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		*4403			-	-	-	-	-	-	-	+	-	-	-	-	-	-
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		-	-	-	-	-	-	-	+	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP™ HLA-B*46 SSP

Product number: 101.544-06 – licensed for PCR
101.544-06u – not licensed for PCR
Lot number: Y24
Expiry date: 2009-September-01
Number of tests: 6
Number of tubes per test: 14

Tube specifications:

Tube No.	Production No.	Tube No.	Production No.
1	2006-232-01	9	2006-232-09
2	2006-232-02	10	2006-232-10
3	2007-379-03	11	2007-379-11
4	2006-232-04	12	2007-379-12
5	2006-232-05	13	2007-379-13
6	2006-232-06	14	2007-379-14
7	2006-232-07		
8	2007-379-08		

The specificity of each primer solution of the HLA-B*46 primer set has been tested against 48 well characterized cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 2, 3, 4, 6, 7, 10, 11 and 12 were available. It was only possible to test the 5'-primer in primer solutions 2 and 7 by separately adding another 3'-primer, the 3'-primer was not possible to test. In primer solutions 4, 6, 10, 11 and 12 it was possible to test both the 5'-primer and the 3'-primer. In primer solution 4 it was only possible to test the 3'-primer, the 5'-primer was not possible to test.

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

Date of approval: 2007-December-15

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: Olerup SSP™ HLA-B*46
Product number: 101.544-06, 101.544-06u
Lot number: Y24

Intended use: HLA-B*46 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-15

Olle Olerup
Managing Director

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-B*46
101.544-06 – licensed for PCR
101.544-06u – not licensed for PCR
Lot No.: **Y24**

24

www.olerup.com

ADDRESSES:

Manufacturer:

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

Tel: +46-8-717 88 27

Fax: +46-8-717 88 18

E-mail: info-ssp@olerup.com

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

Distributed by:

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

Tel: +43-1-710 15 00

Fax: +43-1-710 15 00 10

E-mail: support-at@olerup.com

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

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

Tel: 1-877-OLERUP1

Fax: 610-344-7989

E-mail: info.us@olerup.com

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

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