

Olerup SSP™ HLA-B*27

Product number: 101.521-24/04 – licensed for PCR
101.521-24u/04u - not licensed for PCR
Lot number: X94
Expiry date: 2009-June-01
Number of tests: 24 test – Product No. 101.521-24
4 tests – Product No. 101.521-04
Number of tubes per test: 24
Storage - pre-aliquoted primers: dark at -20°C
- PCR Master Mix: -20°C

This Product Description is only valid for Lot No. X94.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP™ HLA-B*27 LOT

The HLA-B*27 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP™* HLA-B*27 lot was made (**Lot No. V40**).

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

Tube	5'-primer	3'-primer	rationale
20	-	Added	Primer added for the B*2736 allele.
22	-	Added	Primer added for the B*2737 allele.
24	Added	Added	Primer pair added for separating the B*2729 and B*3702 alleles.

Change in revision R01 compared to R00:

1. Lot number on Certificate of Analyses on page 28 corrected to X94.

Change in revision R02 compared to R01:

2. Expiration date on page 28 corrected.

Change in revision R03 compared to R02:

3. The B*270504 allele is not amplified by primer mix 6.

PRODUCT DESCRIPTION

HLA-B*27 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*2701 to B*2738 alleles.

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

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

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

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

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

PLATE LAYOUT

Each test consists of 24 PCR reactions in a 24 well 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

The 24 well cut PCR plate is marked with 'B*27 X94'.

Well No. 1 is marked with '1'.

The PCR plates are covered with a PCR-compatible foil.

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

INTERPRETATION

The interpretation of HLA-B*27 SSP subtypings will be influenced by thirteen B*07, three B*08, most B*13, sixteen B*15, the B*18, five B*35, the B*37, most B*40, eight B*44, the B*47, most B*48, four B*51, the B*5208, four B*55, two B*57, the B*81 and the B*82 alleles when present on the other haplotype. In addition, the Cw*0334 allele will be amplified by primer mix 16 and the primers in vial 10 will weakly amplify the Cw*0705 allele.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*27 alleles, i.e. **B*2701 to B*2738**, recognized by the HLA Nomenclature Committee in July 2007¹ will give rise to unique amplification patterns by the primers in the HLA-B*27 subtyping kit.

The HLA-B*27 subtyping kit cannot distinguish the B*270502 to the B*270510 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-July-09, release 2.18.0
www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 37 HLA-B*27 alleles can be combined in 703 homozygous and heterozygous combinations. 277 of these genotypes do not give rise to unique amplification patterns. (The different lengths of the specific PCR products in lanes 14, 18 to 20, 22 and 24 were not considered in these calculations.)

++++++	+-+---	-----	2707,2718 = 2718,2727
++++++	-----	-----	2717,2718 = 2718,2734
++++++	-----	--+---	2718,2735 = 2718,2738
++++++	-----	-----	2705,2718 = 2710,2718 = 2718,2728 = 2718,2729
+++++-	--+-----	-----	2707,2723 = 2723,2727
+++++-	-----	-----	2717,2723 = 2723,2734
+++++-	-----	-----	2721,2729 = 2723,2729
+++++-	-----	--+---	2723,2735 = 2723,2738
+++-+-	--+-----	-----	2707,2729 = 2727,2729
+++-+-	--+-----	-----	2701,2707 = 2701,2727
+++-+-	-----	-----	2717,2729 = 2729,2734
+++-+-	-----	-----	2701,2717 = 2701,2734
+++-+-	-----	--+---	2729,2735 = 2729,2738
+++-+-	-----	-----	2701,2729 = 2705,2729 = 2728,2729 = 2729,2729
+++-+-	-----	--+---	2701,2735 = 2701,2738
++++++	+-+---	-----	2711,2718 = 2718,2720
++++++	-----	-----	2718,2731 = 2718,2737
++++++	-----	-----	2701,2718 = 2715,2718 = 2718,2721 = 2718,2723
++++++	--+-----	-----	2701,2733 = 2723,2733
++++++	-----	-----	2701,2712 = 2712,2723
++++++	-----	-----	2701,2726 = 2723,2726
++++++	-----	-----	2701,2708 = 2708,2723
+++-+-	++-----	-----	2701,2706 = 2706,2723
+++-+-	+-+-----	-----	2701,2720 = 2720,2723
+++-+-	+-+-----	-----	2701,2704 = 2704,2723
+++-+-	+-+-----	-----	2701,2715 = 2715,2723
+++-+-	--+-----	-----	2701,2724 = 2723,2724
+++-+-	--+-----	-----	2701,2711 = 2711,2723
+++-+-	-----	--+---	2701,2725 = 2723,2725
+++-+-	-----	-----	2701,2736 = 2723,2736
+++-+-	-----	-----	2701,2731 = 2723,2731 = 2723,2737
+++-+-	-----	-----	2701,2721 = 2701,2723 = 2721,2723 = 2723,2723

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+---+--+	+-----+	-----++	2708,2718	=	2718,2718
-+-+--+	--+-----	-----+-	2702,2707	=	2702,2727
-+-+--+	-----+-	-----+-	2702,2717	=	2702,2734
-+-+--+	-----+	-----+-	2702,2719	=	2705,2730
-+-+--+	-----	--+-----	2702,2735	=	2702,2738
--++--+	--+-----	-----+-	2703,2707	=	2703,2727
--++--+	-----+-	-----+-	2703,2717	=	2703,2734
--++--+	-----	--+-----	2703,2735	=	2703,2738
--++--+	-----	-----+-	2703,2703	=	2703,2705
---++++	--+-----	-----+-	2707,2712	=	2712,2727 = 2716,2733
---++++	--+-----	-----+-	2707,2726	=	2726,2727
---++++	--+-----	-----+-	2705,2733	=	2707,2708 = 2708,2727
---++++	-----+	-----+-	2712,2717	=	2712,2734
---++++	-----+	--+-----	2712,2735	=	2712,2738
---++++	-----+	-----+-	2705,2712	=	2708,2716 = 2712,2716
---++++	-----+	-----+-	2717,2726	=	2726,2734
---++++	-----+	-----+-	2708,2717	=	2708,2734
---++++	-----	--+-----	2726,2735	=	2726,2738
---++++	-----	--+-----	2708,2735	=	2708,2738
---+++++	++-----	-----+-	2705,2706	=	2706,2710
---+++++	++-----	-----+-	2704,2707	=	2704,2727
---+++++	++-----	-----+-	2705,2720	=	2710,2711 = 2710,2720 = 2715,2727
---+++++	+-----+	-----+-	2704,2717	=	2704,2734
---+++++	+-----	--+-----	2704,2735	=	2704,2738
---+++++	+-----	--+-----	2710,2725	=	2715,2735
---+++++	+-----	-----+-	2709,2715	=	2710,2736
---+++++	+-----	-----+-	2704,2705	=	2704,2710
---+++++	+-----	-----+-	2705,2715	=	2710,2715 = 2710,2721
---+++++	--+-----	--+-----	2707,2725	=	2711,2738
---+++++	--+-----	-----+-	2707,2736	=	2727,2736
---+++++	--+-----	-----+-	2707,2731	=	2727,2731
---+++++	-----+	-----+-	2717,2736	=	2734,2736
---+++++	-----+	-----+-	2717,2731	=	2731,2734
---+++++	-----	--+-----	2709,2725	=	2735,2736 = 2736,2738
---+++++	-----	--+-----	2731,2735	=	2731,2738
---+++++	-----	--+-----	2705,2725	=	2725,2735
---+++++	-----	--+-----	2721,2738	=	2725,2738
---+++++	-----	-----+-	2705,2736	=	2709,2736
---++++-	+++-----	-----+-	2706,2707	=	2706,2727
---++++-	++-----	-----+-	2707,2720	=	2720,2727
---++++-	--+-----	-+-----	2707,2724	=	2711,2732 = 2724,2732
---++++-	--+-----	-----+-	2711,2727	=	2721,2727
---++++-	--+-----	-----	2707,2711	=	2707,2721
---+--+	++-----	-----+-	2707,2710	=	2710,2727
---+--+	+-----+	-----+-	2710,2717	=	2710,2734
---+--+	+-----	--+-----	2710,2735	=	2710,2738
---+--+	+-----	-----+-	2705,2710	=	2710,2710
---+--+	--+-----	-----+-	2707,2713	=	2713,2727
---+--+	--+-----	-----+-	2707,2716	=	2716,2727
---+--+	--+-----	-----+-	2707,2717	=	2717,2727
---+--+	--+-----	-----+-	2707,2737	=	2727,2737
---+--+	--+-----	-----+-	2705,2707	=	2705,2727

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---+--+  ---+--+  -----+  2713,2717 = 2713,2734
---+--+  ---+--+  ---+--+  2713,2735 = 2713,2738
---+--+  ---+--+  -----+  2705,2713 = 2713,2713
---+--+  -----+  -----+  2716,2717 = 2716,2734
---+--+  -----+  ---+--+  2716,2735 = 2716,2738
---+--+  -----+  -----+  2705,2716 = 2716,2716
---+--+  -----+  ---+--+  2717,2735 = 2717,2738
---+--+  -----+  -----+  2717,2737 = 2734,2737
---+--+  -----+  -----+  2705,2717 = 2705,2734 = 2717,2717 =
                                2717,2734
---+--+  -----  ---+--+  2735,2737 = 2737,2738
---+--+  -----  ---+--+  2705,2735 = 2705,2738 = 2735,2738
---+--+  ---+--+  ---+--+  2707,2735 = 2727,2735
---+--+  ---+--+  ---+--+  2707,2709 = 2709,2727
---+--+  ---+--+  -----+  2707,2727 = 2727,2727
-----+  ---+--+  -----+  2711,2726 = 2731,2733 = 2733,2737
-----+  -----+  -----+  2712,2731 = 2712,2737
-----+  -----  -----+  2708,2731 = 2708,2737 = 2721,2726 =
                                2726,2731 = 2726,2737
-----+  ---+--+  -----  2711,2733 = 2721,2733
-----+  ++-----  -----+  2706,2731 = 2706,2737
-----+  ++-----  -----+  2704,2706 = 2706,2715
-----+  ++-----  -----+  2704,2711 = 2704,2720
-----+  ++-----  -----+  2720,2731 = 2720,2737
-----+  ++-----  -----+  2704,2731 = 2704,2737
-----+  ++-----  -----+  2704,2704 = 2704,2715 = 2704,2721
-----+  ++-----  -----+  2715,2731 = 2715,2737
-----+  ++-----  -----  2715,2715 = 2715,2721
-----+  ++-----  -+-----  2724,2731 = 2724,2737
-----+  ++-----  -----+  2711,2731 = 2711,2737
-----+  ++-----  ---+--+  2725,2731 = 2725,2737
-----+  ++-----  ---+--+  2721,2725 = 2725,2725
-----+  ++-----  ---+--+  2731,2736 = 2736,2737
-----+  ++-----  ---+--+  2721,2736 = 2736,2736
-----+  ++-----  -----+  2721,2731 = 2721,2737 = 2731,2731 =
                                2731,2737
-----+  ++-----  -----+  2706,2711 = 2706,2720
-----+  ++-----  -----+  2706,2706 = 2706,2721
-----+  ++-----  -----+  2711,2720 = 2720,2720 = 2720,2721
-----+  ++-----  -+-----  2711,2724 = 2721,2724 = 2724,2724
-----+  ++-----  -----  2711,2711 = 2711,2721
-----+  ++-----  -----+  2708,2712 = 2712,2712
-----+  ++-----  -----+  2708,2726 = 2726,2726

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(2705 = 270502 to 270510)

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101.521-24/04 – licensed for PCR.

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101.521-24/04 and 101.521-24u/04u

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GUARANTEE

Olerup SSP AB guarantees that the primers in the HLA-B*27 subtyping kit have specificities given in the Specificity and Interpretation Tables of the product insert and in the GenoVision version of the HELMBERG-SCORETM 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

101.521-24/04 – licensed for PCR

For one HLA-B*27 subtyping, add at room temperature in a 0.5 ml tube:

28 x 2 μl = 56 μl DNA (30 ng/μl)

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

28 x 5 μl = 140 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-H₂O mixture into each of the 24 wells of an HLA-B*27 subtyping. **Well No. 1 of the 24 well PCR plate is marked with '1'**. Close the 24 well PCR plate with the provided lids.

101.521-24u/04u – not licensed for PCR

For one HLA-B*27 subtyping, add at room temperature in a 0.5 ml tube:

28 x 2 μl = 56 μl DNA (30 ng/μl)

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

2.2 μl *Taq* polymerase (5 units/μl)

28 x 5 μl – 2.2 μl = 137.8 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-*Taq*-H₂O mixture into each of the 24 wells of an HLA-B*27 subtyping. **Well No. 1 of the 24 well PCR plate is marked with '1'**. Close the 24 well PCR plate with the provided lids.

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

PCR cycling parameters:

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

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

AGAROSE GEL ELECTROPHORESIS

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

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

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

DOCUMENTATION AND INTERPRETATION

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

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

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

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

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

INTERPRETATION SOFTWARE

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

PCR MASTER MIXES

The PCR Master Mix complete with *Taq* contains:

<i>Taq</i> polymerase	0.4 unit per 10 µl SSP reaction
nucleotides	final concentration of each dNTP is 200 µM
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 µg/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 µM
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 µg/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°C for 4 weeks, but the PCR Master Mixes are then no longer stable for 24 months.

SPECIFICITY TABLE

HLA-B*27 SSP subtyping

Specificities and sizes of the PCR products of the 24 primer mixes used for HLA-B*27 SSP subtyping

Primer Mix	Approx. size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*27 alleles ³	Other amplified HLA Class I alleles ^{3,4}
1	390 bp	800 bp	2701, 2718, 2723, 2729	180101, 180103, 1802, 1804-1815, 1817N-1824, 1826, 3542, 370101-3713, 400201, 400202-400203 [?] , 4003-40060102, 400602 [?] , 4008, 4009-4011 [?] , 4013, 401401-4015 [?] , 4018-4022N [?] , 4024-4038 [?] , 4039, 4040 [?] , 4042 [?] , 4043, 4044-4049 [?] , 4050, 4052-4058 [?] , 4061-4065 [?] , 4067-4069 [?] , 4070, 4071 [?] , 4074-4076 [?] , 47010101-47054013, 4019 [?]
2	405 bp	800 bp	2702, 2730	
3⁵	120 bp	1070 bp	2703	
4	245 bp	1070 bp	2703, 270502-270510, 2707, 2709, 2710, 2713, 2714, 2716, 2717, 2719, 2727-2729, 2732, 2734, 2735, 2738	370101-3704, 3706-3709, 3712, 3713
5	400 bp	1070 bp	270401-270402, 2706, 2708, 2711, 2712, 2715, 2718, 2720, 2721, 2723-2726, 2731, 2733, 2736	180101-1808, 1810-1815, 1817N-1824, 1826, 3542, 400201-400602, 4008, 4009-4011 [?] , 401401-4016 [?] , 4018 [?] , 4020-4022N [?] , 4024-4036 [?] , 4038 [?] , 4039, 4040 [?] , 4042-4046 [?] , 4048-4050 [?] , 4052-4058 [?] ,

				4061-4065 [?] , 4067-4069 [?] , 4070, 4071 [?] , 4074-4076 [?] , 4702, 4703, 8201, 8202
6	415 bp	800 bp	2701-270402, 270502-270503, 270505-2707, 2709-2711, 2713-2717, 2719-2721, 2723-2725, 2727-2732, 2734-2738	1809, 370101-3704, 3706-3710, 3712, 3713, 4013, 4019 [?] , 4047 [?] , 47010101-47010102, 4703-4705
7	410 bp	1070 bp	2708, 2712, 2718, 2726, 2733	180101-1808, 1810-1815, 1817N-1824, 1826, 3542, 3705, 400201-400602, 4008, 4009-4011 [?] , 401401-4015 [?] , 4018 [?] , 4020-4022N [?] , 4024-4038 [?] , 4039, 4040 [?] , 4042-4046 [?] , 4048-4050 [?] , 4052-4058 [?] , 4061-4065 [?] , 4067-4069 [?] , 4070, 4071 [?] , 4074 [?] , 4075 [?] , 4702, 4703 ^{faintly} , 8201, 8202
8⁵	95 bp	1070 bp	2701-270402, 270502-270510, 2708, 2710, 2712, 2713, 2715-2718, 2723, 2725, 2726, 2728, 2729, 2731, 2736-2738	3702, 4704, 4705
9	205 bp	1070 bp	270401-270402, 2706, 2710, 2715, 2718, 2720	
10⁵	85 bp	800 bp	2706	Cw*0705^{weakly}
11	190 bp	1070 bp	2707, 2711, 2720, 2724, 2727, 2733	070501-0706, 0732, 0740, 0753, 1301-130204, 1307N-1309, 1311, 1313-1320, 3531, 400101-400203, 4004, 40060101-4008, 4010-4016, 4019, 4021-4023, 4025, 4027, 4029, 4030,

				4034-4038, 4040, 4043, 4045-4050, 4053-4058, 4061, 4062, 4064-4070, 4072-4077, 4431, 480101-480102, 480301-4807, 4809- 4816, 4818, 5110, 5116, 5131, 5134, 5208, 5509, 5522, 5524, 8101, 8102
12	430 bp	1070 bp	2713	
13⁶	370 bp	800 bp	2712, 2716	
14^{5,8}	120, 345 bp	800 bp	2717, 2734	400602, 570302
15	370 bp	1070 bp	2718, 2729	180101-1806, 1808- 1815, 1817N-1824, 1826, 3520, 370101- 3713, 400201, 400202 [?] , 4003- 40060102, 400602 [?] , 4009-4011 [?] , 401401- 4015 [?] , 4018-4022N [?] , 4024-4038 [?] , 4039, 4040 [?] , 4042-4050 [?] , 4052-4058 [?] , 4062- 4065 [?] , 4067 [?] , 4069 [?] , 4070, 4071 [?] , 4074 [?] , 4076 [?] , 47010101- 4705
16⁷	245 bp	1070 bp	2719, 2730	0712, 0714, 0718, 1301, 1307N, 1313, 1317, 1320, 3515, 3533, 4004, 4030, 4034, 4059, 4064, 4068, 4416, 4421, 4817, 5713, Cw*0334
17⁵	100 bp	1070 bp	2714	4420, 4447
18^{5,9}	95, 175 bp	800 bp	2724, 2732	0728, 0814, 4031, 4045, 4454, 480101- 480102, 4804, 4806, 4807, 4809, 4811, 4815, 4816, 4818, 8101, 8102
19^{5,10}	95, 235 bp	800 bp	2725, 2735, 2738	9529

20^{5,11}	95, 175 bp	800 bp	2709, 2736	
21⁵	110 bp	800 bp	270401, 270402 [?] , 2706	
22¹²	195, 380 bp	800 bp	2726, 2731, 2737	8201, 8202
23	235 bp	800 bp	2701-270402, 270502-2706, 2708-2710, 2712, 2713, 2716-2718, 2720, 2723, 2726, 2727, 2729, 2731, 2735-2737	3702, 4704, 4705
24^{5,13}	105, 260 bp	1070 bp	2718, 2728, 2729	0727, 0750, 0804, 0817, 1503, 1547, 1549, 1554, 1561, 1562, 1564, 1568, 1569, 1591, 1598, 9503, 9523, 9527, 9532, 1802, 3803, 390201-390202, 3908, 391301- 391302, 3923, 3939, 4012, 4046, 4440, 4444, 480101-4805, 4807-4818, 5518

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*27 SSP subtypings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 bp or more. Size differences shorter than 20 bp are not given. For high resolution SSP kits the length of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

PCR fragments longer than the control band may sometimes be observed. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

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

Tube number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*27 subtyping.

In addition, tubes number 2, 6, 10, 13, 14, 18 to 23 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.

³For several HLA-B alleles 1st exon nucleotide sequences are not available. In these instances it is not known whether some of the primers of the HLA-B*27 SSP subtyping kit are completely matched with the target sequences or not. We assume that unknown sequences in the 1st exon are conserved within allelic groups.

⁴Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B*27 alleles will be amplified by primer mixes 1, 2, 4 to 8, 11, 15, to 20, 22 to 24. In addition, the primers in vial 10 will amplify the Cw*0705 allele and the Cw*0334 allele will be amplified by primer mix 16.

⁵Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁶Primer mix 13 has a tendency of giving rise to a primer oligomer artifact.

⁷Primer mix 16 has a tendency of giving rise to nonspecific amplifications.

⁸Primer mix 14: Specific PCR product of 120 bp in the B*2734 and B*400602 and 570302 alleles. Specific PCR product of 345 bp in the B*2717 allele.

⁹Primer mix 18: Specific PCR product of 95 bp in the B*2732 and B*0728, 0814 and 4454 alleles. Specific PCR product of 175 bp in the B*2724 and B*4031, 4045, 480101-480102, 4804, 4806, 4807, 4809, 4811, 4815, 4816, 4818, 8101 and 8102 alleles.

¹⁰Primer mix 19: Specific PCR fragment of 95 bp in the B*2732 allele. Specific PCR fragment of 235 bp in the B*2725, 2738 and B*9529 alleles.

¹¹Primer mix 20: Specific PCR fragment of 95 bp in the B*2709 allele. Specific PCR fragment of 175 bp in the B*2736 allele.

¹²Primer mix 22: Specific PCR fragment of 195 bp in the B*2737 allele. Specific PCR fragment of 380 bp in the B*2726, 2731 and B*8201-8202 alleles.

¹³Primer mix 24: Specific PCR fragment of 105 bp in the B*2718, 2729 and the B*0727, 0750, 0804, 0817, 1503, 1547, 1549, 1554, 1561, 1562, 1564, 1568, 1569, 1591, 1598, 3803, 390201-390202, 3908, 391301-391302, 3923, 3939, 4012, 4046, 4440, 4444, 480101-4805, 4807-4818, 5518, 9503, 9523, 9527 and 9532 alleles. Specific PCR fragment of 260 bp in the B*2728 and B*1802 alleles.

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

HLA-B*27

101.521-24/04 – licensed for PCR

101.521-24u/04u – not licensed for PCR

Lot No.: **X94**

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INTERPRETATION TABLE												
HLA-B*27 SSP subtyping												
Amplification patterns of the B*2701 to B*2738 alleles												
	Tube ⁵											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	390	405	120	245	400	415	410	95	205	85	190	430
PCR product												
Length of int.	800	800	1070	1070	1070	800	1070	1070	1070	800	1070	1070
pos. control ¹												
5'-primer(s) ²	72	72	167	97	72	72	72	363	363	363	412	14
	5'-gCT ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	5'-TCC ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	5'-AAT ^{3'}	5'-AAT ^{3'}	5'-AAT ^{3'}	5'-ATA ^{3'}	5'-ggA ^{3'}
3'-primer(s) ³	292	309	247	301	301	317	311	418	527	409	559	272
	5'-gTA ^{3'}	5'-ATC ^{3'}	5'-ATg ^{3'}	5'-gTC ^{3'}	5'-gCT ^{3'}	5'-ggA ^{3'}	5'-ggT ^{3'}	5'-gTC ^{3'}	5'-CCT ^{3'}	5'-ATA ^{3'}	5'-CTC ^{3'}	5'-TgC ^{3'}
					302							
					5'-ggC ^{3'}							
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-B allele ⁴												
*2701	+					+		+				
*2702		+				+		+				
*2703			+	+		+		+				
*270401					+	+		+	+			
*270402					+	+		+	+			
*270502-270503, 270505-270510				+		+		+				
*270504				+				+				
*2706					+	+			+	+		
*2707				+		+					+	
*2708					+		+	+				
*2709				+		+						
*2710				+		+		+	+			
*2711					+	+					+	
*2712					+		+	+				
*2713				+		+		+				+
*2714				+		+						
*2715					+	+		+	+			
*2716				+		+		+				
*2717				+		+		+				
*2718	+				+		+	+	+			
*2719				+		+						
*2720					+	+			+		+	
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE												
HLA-B*27 SSP subtyping												
Amplification patterns of the B*2701 to B*2738 alleles												
Tube ⁵												
13	14	15	16	17	18	19	20	21	22	23	24	
370	120	370	245	100	95	95	95	110	195	235	105	Length of spec.
	345				175	235	175		380		260	PCR product
800	800	1070	1070	1070	800	800	800	800	800	800	1070	Length of int.
												pos. control ¹
72	72	72	355	357	363	363	363	704	72	363	206	5'-primer(s) ²
5'-gCT ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	5'-TCA ^{3'}	5'-Tgg ^{3'}	5'-AgC ^{3'}	5'-AAT ^{3'}	5'-AAT ^{3'}	5'-Tgg ^{3'}	5'-gCT ^{3'}	5'-AAT ^{3'}	5'-AgA ^{3'}	
	409										363	
	5'-ggC ^{3'}										5'-AAT ^{3'}	
272	248	272	559	418	418	419	418	774	97	559	272	3'-primer(s) ³
5'-TgC ^{3'}	5'-AAA ^{3'}	5'-Tgg ^{3'}	5'-CTC ^{3'}	5'-gTC ^{3'}	5'-gTC ^{3'}	5'-CgA ^{3'}	5'-gTg ^{3'}	5'-ggT ^{3'}	5'-gTA ^{3'}	5'-CTC ^{3'}	5'-Tgg ^{3'}	
	486				499	559	499		280		583	
	5'-gCT ^{3'}				5'-ggA ^{3'}	5'-CAg ^{3'}	5'-ggA ^{3'}		5'-CTg ^{3'}		5'-gTg ^{3'}	
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.
												HLA-B allele ⁴
										+		*2701
										+		*2702
										+		*2703
								+		+		*270401
								?		+		*270402
										+		*270502-270503, 270505-270510
										+		*260504
								+		+		*2706
										+		*2707
										+		*2708
							+			+		*2709
										+		*2710
												*2711
+										+		*2712
										+		*2713
				+								*2714
												*2715
+										+		*2716
	+									+		*2717
		+								+	+	*2718
			+									*2719
										+		*2720
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.

Length of spec.	390	405	120	245	400	415	410	95	205	85	190	430
PCR product												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
*2721					+	+						
*2723	+				+	+		+				
*2724					+	+					+	
*2725					+	+		+				
*2726					+		+	+				
*2727				+		+					+	
*2728				+		+		+				
*2729	+			+		+		+				
*2730		+				+						
*2731					+	+		+				
*2732				+		+						
*2733					+		+				+	
*2734				+		+						
*2735				+		+						
*2736					+	+		+				
*2737						+		+				
*2738				+		+		+				
*070501-0706, 0732, 0740, 0753, 130201- 130204, 1308, 1309, 1311, 1314-1316, 1318, 1319, 3531, 400101-400105, 4007, 4023, 4066, 4072, 4073, 4077, 4431, 5110, 5116, 5131, 5134, 5208, 5509, 5522, 5524											+	
*0712, 0714, 0718, 3515, 3533, 4059, 4416, 4421, 5713												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12

370	120	370	245	100	95	95	95	110	195	235	105	Length of spec. PCR product
	345				175	235	175		380		260	
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.
												*2721
										+		*2723
					+							*2724
						+						*2725
									+	+		*2726
										+		*2727
											+	*2728
		+								+	+	*2729 ⁶
			+									*2730
									+	+		*2731
					+							*2732
												*2733
	+											*2734
						+				+		*2735
							+			+		*2736
									+	+		*2737
						+						*2738
												*070501-0706, 0732, 0740, 0753, 130201- 130204, 1308, 1309, 1311, 1314-1316, 1318, 1319, 3531, 400101-400105, 4007, 4023, 4066, 4072, 4073, 4077, 4431, 5110, 5116, 5131, 5134, 5208, 5509, 5522, 5524
			+									*0712, 0714, 0718, 3515, 3533, 4059, 4416, 4421, 5713
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.

Length of spec.	390	405	120	245	400	415	410	95	205	85	190	430
PCR product												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
*0727, 0750, 0804, 0817, 1503, 1547, 1549, 1554, 1561, 1562, 1564, 1568, 1569, 1591, 1598, 3803, 390201- 390202, 3908, 391301-391302, 3923, 3939, 4440, 4444, 4802, 4808, 5518, 9503, 9523, 9527, 9532												
*0728, 0814, 4454												
*1301, 1307N, 1313, 1317, 1320											+	
*180101, 180103, 1804-1806, 1808, 1810-1815, 1817N- 1824, 1826, 4003, 4005, 4039, 4702	+				+		+					
*180102, 1803					+		+					
*1802	+				+		+					
*1807, 3542	+				+		+					
*1809, 3710, 47010101-47010102	+					+						
*3520												
*370101-370104, 3703N, 3704, 3706- 3709, 3712, 3713	+			+		+						
*3702	+			+		+		+				
*3705	+						+					
*3711	+											
*400201, 40060101- 40060102, 4070	+				+		+				+	
*400202	?				+		+				+	
*400203	?				+		+				+	
*4004	+				+		+				+	
*400602	?				+		+				+	
*4008	+				+		+				+	
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12

370	120	370	245	100	95	95	95	110	195	235	105	Length of spec.
	345				175	235	175		380		260	PCR product
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.
											+	*0727, 0750, 0804, 0817, 1503, 1547, 1549, 1554, 1561, 1562, 1564, 1568, 1569, 1591, 1598, 3803, 390201- 390202, 3908, 391301-391302, 3923, 3939, 4440, 4444, 4802, 4808, 5518, 9503, 9523, 9527, 9532
					+							*0728, 0814, 4454
			+									*1301, 1307N, 1313, 1317, 1320
		+										*180101, 180103, 1804-1806, 1808, 1810-1815, 1817N- 1824, 1826, 4003, 4005, 4039, 4702
		+										*180102, 1803
		+									+	*1802
												*1807, 3542
		+										*1809, 3710, 47010101-47010102
		+										*3520
		+										*370101-370104, 3703N, 3704, 3706- 3709, 3712, 3713
		+								+		*3702 ⁶
		+										*3705
		+										*3711
		+										*400201, 40060101- 40060102, 4070
		?										*400202
												*400203
		+	+									*4004
	+	?										*400602
												*4008
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.

Length of spec.	390	405	120	245	400	415	410	95	205	85	190	430
PCR product												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
*4009, 4018, 4020, 4024, 4026, 4028, 4032, 4033, 4042, 4044, 4052, 4063, 4071	?				?		?					
*4010, 4011, 401401-4015, 4021, 4022N, 4025, 4027, 4029, 4035, 4036, 4038, 4040, 4048, 4049, 4053-4058, 4062, 4065, 4067, 4069, 4074	?				?		?				+	
*4012, 480301-480302, 4805, 4810, 4812-4814											+	
*4013	+	+				+					+	
*4016					?						+	
*4019	?	?				?					+	
*4030, 4034, 4064	?				?		?				+	
*4031	?				?		?					
*4037	?						?				+	
*4043, 4050	+				?		?				+	
*4045	?				?		?				+	
*4046	?				?		?				+	
*4047	?					?					+	
*4061, 4075	?				?		?				+	
*4068	?				?		?				+	
*4076	?				?						+	
*4420, 4447												
*4703	+				+	+	f					
*4704, 4705	+					+		+				
*480101-480102, 4804, 4807, 4809, 4811, 4815, 4816, 4818											+	
*4806, 8101, 8102											+	
*4817												
*570302												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12

370	120	370	245	100	95	95	95	110	195	235	105	Length of spec.
	345				175	235	175		380		260	PCR product
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.
		?										*4009, 4018, 4020, 4024, 4026, 4028, 4032, 4033, 4042, 4044, 4052, 4063, 4071
		?										*4010, 4011, 401401- 4015, 4021, 4022N, 4025, 4027, 4029, 4035, 4036, 4038, 4040, 4048, 4049, 4053-4058, 4062, 4065, 4067, 4069, 4074
											+	*4012, 480301- 480302, 4805, 4810, 4812-4814
												*4013
												*4016
		?										*4019
		?	+									*4030, 4034, 4064
		?			+							*4031
		?										*4037
		?										*4043, 4050
		?			+							*4045
		?									+	*4046
		?										*4047
												*4061, 4075
			+									*4068
		?										*4076
				+								*4420, 4447
		+										*4703
		+								+		*4704, 4705
					+						+	*480101-480102, 4804, 4807, 4809, 4811, 4815, 4816, 4818
					+							*4806, 8101, 8102
	+		+								+	*4817
												*570302
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.

Length of spec.	390	405	120	245	400	415	410	95	205	85	190	430
PCR product												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
*8201, 8202					+		+					
*9529												
HLA-B allele ⁴												
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12
Cw*0334												
Cw*0705										W		
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12

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*27 subtyping. In addition, tubes number 2, 6, 10, 13, 14, 18 to 23 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

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

²The nucleotide position, in the 1st, 2nd, 3rd or 4th 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, 3rd or 4th 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.

370	120	370	245	100	95	95	95	110	195	235	105	Length of spec.
	345				175	235	175		380		260	PCR product
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.
									+			*8201, 8202
						+						*9529
												HLA-B allele⁴
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.
			+									Cw*0334
												Cw*0705
13	14	15	16	17	18	19	20	21	22	23	24	Tube No.

⁴The sequence of the B*270501 allele has been shown to be identical to B*270502.

The sequence of the B*2722 allele has been shown to be identical to the corrected B*2706 nucleotide sequence.

⁵Primer mix 14: Specific PCR product of 120 bp in the B*2734 and B*400602 and 570302 alleles. Specific PCR product of 345 bp in the B*2717 allele.

Primer mix 18: Specific PCR product or 95 bp in the B*2732 and B*0728, 0814 and 4454 alleles. Specific PCR product of 175 bp in the B*2724 and B*4031, 4045, 480101-480102, 4804, 4806, 4807, 4809, 4811, 4815, 4816, 4818, 8101 and 8102 alleles.

Primer mix 19: Specific PCR fragment of 95 bp in the B*2732 allele. Specific PCR fragment of 235 bp in the B*2725, 2738 and B*9529 alleles.

Primer mix 20: Specific PCR fragment of 95 bp in the B*2709 allele. Specific PCR fragment of 175 bp in the B*2736 allele.

Primer mix 22: Specific PCR fragment of 195 bp in the B*2737 allele. Specific PCR fragment of 380 bp in the B*2726, 2731 and B*8201-8202 alleles.

Primer mix 24: Specific PCR fragment of 105 bp in the B*2718, 2729 and the B*0727, 0750, 0804, 0817, 1503, 1547, 1549, 1554, 1561, 1562, 1564, 1568, 1569, 1591, 1598, 3803, 390201-390202, 3908, 391301-391302, 3923, 3939, 4012, 4046, 4440, 4444, 480101-4805, 4807-4818, 5518, 9503, 9523, 9527 and 9532 alleles. Specific PCR fragment of 260 bp in the B*2728 and B*1802 alleles.

'w', might be weakly amplified.

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

CELL LINE VALIDATION SHEET																				
HLA-B*27 SSP subtyping kit																				
					Tube															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.:	200620501	200620502	200620503	200620504	200620505	200620506	200620507	200620508	200620509	200620510	200620511	200735212	200620513	200620514	200620515	200620516
	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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CELL LINE VALIDATION SHEET													
HLA-B*27 SSP subtyping kit													
					Tube								
					17	18	19	20	21	22	23	24	
				Prod. No.:	200620517	200620518	200620519	200735220	200620521	200735222	200620523	200735224	
	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 SSPTM HLA-B*27 SSP

Product number: 101.521-24/04 – licensed for PCR
101.521-24u/04u – not licensed for PCR
Lot number: X94
Expiry date: 2009-June-01
Number of tests: 24 test – Product No. 101.521-24
4 tests – Product No. 101.521-04
Number of tubes per test: 24

Tube specifications:

Tube No.	Production No.	Tube No.	Production No.	Tube No.	Production No.
1	2006-205-01	9	2006-205-09	17	2006-205-17
2	2006-205-02	10	2006-205-10	18	2006-205-18
3	2006-205-03	11	2006-205-11	19	2006-205-19
4	2006-205-04	12	2007-352-12	20	2007-352-20
5	2006-205-05	13	2006-205-13	21	2006-205-21
6	2006-205-06	14	2006-205-14	22	2007-352-22
7	2006-205-07	15	2006-205-15	23	2006-205-23
8	2006-205-08	16	2006-205-16	24	2007-352-24

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 3, 12, 13, 14, 16, 17, 19, 20, 22 and 24 were available. The specificities of the primers in primer solutions 13, 16, 17, 19, 20, 22 and 24 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. Additional 3'-primers in primer solution 5 was tested by separately adding one additional 5'-primer. In primer solutions 3 and 14 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solution 12 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-August-21

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: Olerup SSP™ HLA-B*27
Product number: 101.521-24/04, 101.521-24u/04u
Lot number: X94

Intended use: HLA-B*27 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-August-21

Olle Olerup
Managing Director

HLA-B*27

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101.521-24/04 – licensed for PCR

101.521-24u/04u – not licensed for PCR

Lot No.: **X94**

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WARRANTY

Olerup SSP AB warrants its products to the original purchaser against defects in materials and workmanship under normal use and application. *Olerup* SSP AB's sole obligation under this warranty shall be to replace, at no charge, any product that does not meet the performance standards stated on the product specification sheet.

This warranty applies only to products that have been handled and stored in accordance with *Olerup* SSP AB's recommendations, and does not apply to products that have been the subject of alternation, misuse, or abuse.

All claims under this warranty must be directed to *Olerup* SSP AB in writing and must be accompanied by a copy of the purchaser's invoice. This warranty is in lieu of all other warranties, expressed or implied, including the warranties of merchantability and fitness for a particular purpose. In no case shall *Olerup* SSP AB be liable for incidental or consequential damages.

This product may not be reformulated, repacked or resold in any form without the written consent of *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

Handle all samples as if capable of transmitting disease. All work should be performed wearing gloves and appropriate protection.

Olerup SSPTM is a trademark of *Olerup* SSP AB.
PCRTM is a trademark of F. Hoffmann-La Roche Ltd.
ARMSTM is a trademark of Zeneca Ltd.

HLA-B*27
101.521-24/04 – licensed for PCR
101.521-24u/04u – not licensed for PCR
Lot No.: **X94**

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www.olerup.com

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

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