Olerup SSP™ HLA-B*27 – unit dose

Product number:	**101.531-48 – licensed for PCR** **101.531-48u – not licensed for PCR**
Lot number:	**Y02**
Expiry date:	**2009-June-01**
Number of tests:	**48**
Number of tubes per test:	**2**
Storage - pre-aliquoted primers:	**dark at -20°C**
- PCR Master Mix:	**-20°C**
- Control DNAs:	**-20°C**

This Product Description is only valid for Lot No. Y02.

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

The HLA-B*27 specificity and interpretation tables has been updated for the HLA-B alleles described since the previous *Olerup* SSP™ HLA-B*27 lot **(Lot No. X18)** was made.

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 yield of specific PCR product.

PRODUCT DESCRIPTION

HLA-B*27 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-B27 specificity, B*2701 to B*2738.
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.
Positive and negative control DNAs are included in the kit.
DNA 1; a B*27-positive DNA as a positive control, **IHW 9315, CML, B*0801,270502.**
DNA 2; a B*73-positive DNA as a negative control, **IHW 9280, LK707, B*520101,7301**. (A B*7301-positive DNA was chosen as negative control, as this is most similar to the B*27 group of alleles in the primer matching regions.)
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.
2 PCR reactions with a reaction volume of 10 µl is performed per sample.
<u>Note:</u> 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 2 PCR reactions. 4 tests are aliquoted in each cut 8 well PCR plate.

1	**2**	**1**	**2**	**1**	**2**	**1**	**2**

The 8 well cut PCR plate is marked with 'HLA-B*27'.
Well No. 1 is marked with the Lot No. 'Y02'.
The PCR plates are covered with a PCR-compatible foil.
Please note: When removing each 8 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

In addition to the HLA-B*27 alleles, the B*3702, B*4704 and B*4705 will be amplified by primer mix 2 of the HLA-B*27 kit.

UNIQUELY IDENTIFIED ALLELES

All the B*27 alleles, i.e. **B*2701 to B*2738**, recognized by the HLA Nomenclature Committee in July 2007[1] are identified by the primers in the HLA-B*27 SSP kit.
In addition, the B*3702, B*4704 and B*4705 alleles are amplified by primer mix 2 of the HLA-B*27 kit.

[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.

LICENSES

101.531-48 – 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.

101.531-48u – <u>not</u> licensed for PCR.
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101.531-48 and 101.531-48u

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*27 SSP 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* is stable for 24 months from the date of manufacture.

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/ul.
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_2O.

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

101.531-48 – licensed for PCR

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

4 x 2 µl = 8 µl DNA (30 ng/µl)
4 x 3 µl = 12 µl PCR Master Mix complete with *Taq* – mix well before taking your aliquot
4 x 5 µl = 20 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-H_2O mixture into each of the 2 wells of an HLA-B*27 typing. ***The 8 well PCR plate is marked with the lot number.*** Close the 8 well PCR plate with the provided lids.

101.531-48u – <u>not</u> licensed for PCR

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

4 x 2 µl = 8 µl DNA (30 ng/µl)
4 x 3 µl = 12 µl PCR Master Mix without *Taq* – mix well before taking your aliquot
0.3 µl *Taq* polymerase (5 units/µl)
4 x 5 µl – 0.3 µl = 19.7 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix-*Taq*-H_2O mixture into each of the 2 wells of an HLA-B*27 typing. ***The 8 well PCR plate is marked with the lot number.*** Close the 8 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^oC.

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 length of the specific PCR product is helpful in the interpretation of the results.

Record the presence of the internal positive control bands.

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

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

PCR MASTER MIXES

The PCR Master Mix complete with *Taq* contains:

Taq 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 typing

Specificity and size of the PCR product of the two primer mixes used for HLA-B*27 SSP typing.

Primer Mix	Approx. size of spec. PCR product[1]	Size of control band[2]	Amplified HLA-B*27 alleles	Other amplified HLA-B alleles[3]
1	145 bp	**430 bp**	2701-270402, 270502-270508, 270510, 2706-2711, 2713-2715, 2717, 2719-2721, 2724, 2725, 2727, 2728, 2730, 2732-2738	
2[4]	95 bp	515 bp	2701-270402, 270502-270510, 2708, 2710, 2712, 2713, 2715-2718, 2723, 2725, 2726, 2728, 2729, 2731, 2736-2738	3702, 4704, 4705

[1]Alleles are assigned by the presence of specific PCR product.
Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen.
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.
[2]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 430 base pairs or a band of 515 base pairs.
Tube number 1 contains the primer pair giving rise to the shorter, 430 bp, internal positive control band in order to help in the correct orientation of the HLA-B*27 typing.
In the presence of a specific amplification the intensity of the control band often decreases.
[3]Due to the sharing of sequence motifs between HLA-B alleles three non-HLA-B*27 alleles will be amplified by primer mix 2.
[4]Short specific PCR fragments are less intense and not as sharp as longer specific bands.

INTERPRETATION TABLE			
HLA-B*27 SSP typing			
Amplification pattern of the B*2701 to 2738 alleles[1]			
	Tube		
	1	**2**	
Length of spec. PCR product	**145**	**95**	**Length of spec. PCR product**
Length of int. pos. control[2]	**430**	**515**	**Length of int. pos. control**
5'-primer[3]	**167**	**363**	**5'-primer[3]**
	5'-gCT3'	**5'-AAT3'**	
3'-primer[4]	**272**	**418**	**3'-primer[4]**
	5'-TgC3'	**5'-gTC3'**	
Tube No.	**1**	**2**	**Tube No.**
HLA-B allele[5]			**HLA-B allele[5]**
***2701-270402, 270502-270508, 270510, 2708, 2710, 2713, 2715, 2717, 2725, 2728, 2736-2738**	**+**	**+**	***2701-270402, 270502-270508, 270510, 2708, 2710, 2713, 2715, 2717, 2725, 2728, 2736-2738**
***2706, 2707, 2709, 2711, 2714, 2719-2721, 2724, 2727, 2730, 2732-2735**	**+**		***2706, 2707, 2709, 2711, 2714, 2719-2721, 2724, 2727, 2730, 2732-2735**
***270509, 2712, 2716, 2718, 2723, 2726, 2729, 2731, 3702, 4704, 4705**		**+**	***270509, 2712, 2716, 2718, 2723, 2726, 2729, 2731, 3702, 4704, 4705**
HLA-B allele[5]			**HLA-B allele[5]**
Tube No.	**1**	**2**	**Tube No.**

[1]Due to the sharing of sequence motifs between HLA-B alleles three non-HLA-B*27 alleles will be amplified by primer mix 2; B*3702, B*4704, B*4705.

[2]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 430 base pairs or a band of 515 base pairs.
Tube number 1 contains the primer pair giving rise to the shorter, 430 bp, internal positive control band in order to help in the correct orientation of the HLA-B*27 typing.
In the presence of a specific amplification the intensity of the control band often decreases.

[3]The nucleotide position, in the 2nd and 3rd exons, 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.

[4]The nucleotide position, in the 2nd and 3rd exons, 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.

[5]The sequence of the B*270501 allele has been shown to be identical to B*270502.
The B*2722 sequence shown to be identical to the corrected B*2706 sequence.

CELL LINE VALIDATION SHEET

HLA-B*27 unit dose SSP kit

				Tube		
					1	2
				Production No.	200735801	200735802
	cell line		HLA-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*27 SSP – unit dose

Product number:	**101.531-48 – licensed for PCR** **101.531-48u – not licensed for PCR**
Lot number:	**Y02**
Expiry date:	**2009-June-01**
Number of tests:	**48**
Number of tubes per test:	**2**

Tube specifications:

Tube No.	Production No.
1	2007-358-01
2	2007-358-02

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

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

Date of approval: 2007-August-29

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP™ HLA-B*27 - unit dose
Product number: 101.531-48/101.531-48U
Lot number: Y02

Intended use: HLA-B*27 low resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Hasselstigen 1
SE-133 33 Saltsjöbaden, Sweden
Phone: +46-8-717 88 27
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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-29

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.

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