

101.531-48 – including *Taq* pol., IFU-01101.531-48u – without *Taq* pol., IFU-02Visit [www.olerup-ssp.com](http://www.olerup-ssp.com) for

“Instructions for Use” (IFU)

Lot No.: **65S**

Lot-specific information

[www.olerup-ssp.com](http://www.olerup-ssp.com)**Olerup SSP<sup>®</sup> HLA-B\*27 – unit dose**

Product number:	101.531-48 – including <i>Taq</i> polymerase 101.531-48u – without <i>Taq</i> polymerase
Lot number:	65S
Expiry date:	2016-January-01
Number of tests:	48
Number of wells per test:	2
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C
- Control DNAs:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

**This Product Description is only valid for Lot No. 65S.**

**CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP<sup>®</sup>*  
HLA-B\*27 Lot (39R)**

The Lot-specific information for HLA-B\*27 unit dose including and without *Taq* polymerase is described in one common Product Insert.

The HLA-B\*27 specificity and interpretation tables has been updated for the HLA-B alleles described since the previous *Olerup SSP<sup>®</sup>* HLA-B\*27 lot (**Lot No. 39R**) was made.

The HLA-B\*27 unit dose primer set is unchanged compared to the previous lot.

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[www.olerup-ssp.com](http://www.olerup-ssp.com)**PRODUCT DESCRIPTION****HLA-B\*27 SSP typing****CONTENT**

The primer set contains 5'- and 3'-primers for identifying the HLA-B27 specificity, B\*27:01 to B\*27:105.

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\*08:01,27:05:02.**

DNA 2; a B\*73-positive DNA as a negative control, **IHW 9280, LK707, B\*52:01:01,73:01.** (A B\*73:01-positive DNA was chosen as negative control, as this is most similar to the B\*27 group of alleles in the primer matching regions.)

**PLATE LAYOUT**

Each test consists of 2 PCR reactions. 4 tests are aliquoted in each cut 8 well PCR plate.

<b>1</b>	<b>2</b>	<b>1</b>	<b>2</b>	<b>1</b>	<b>2</b>	<b>1</b>	<b>2</b>
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The 8 well cut PCR plate is marked with 'B27' in silver/gray ink.

Well No. 1 is marked with the Lot No. '65S'.

A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.

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\*44:97 allele is amplified by primer mix 1 and the B\*37:02, B\*47:04-47:05 and B\*48:26 alleles are amplified by primer mix 2 of the HLA-B\*27 kit.

**UNIQUELY IDENTIFIED ALLELES**

All the HLA-B\*27 alleles, i.e. **B\*27:01 to B\*27:105**, recognized by the HLA Nomenclature Committee in April 2013<sup>1</sup> are identified by the primers in the HLA-B\*27 SSP kit.

In addition, the B\*44:97 is amplified by primer mix 1 and the B\*37:02, B\*47:04-47:05 and B\*48:26 alleles are amplified by primer mix 2 of the HLA-B\*27 kit.

<sup>1</sup>HLA-B alleles listed on the IMGT/HLA web page 2013-April-17, release 3.12.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

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[www.olerup-ssp.com](http://www.olerup-ssp.com)**PROTOCOL****DNA EXTRACTION**

Extracted, highly pure DNA is needed for SSP typings. DNA samples to be used for PCR-SSP HLA typing should be re-suspended in dH<sub>2</sub>O. The A260/A280 ratio should be 1.6 – 2.0 by UV spectrophotometry for optimal band visualization during electrophoresis.

We recommend automated DNA extraction with the QIAGEN EZ1 DSP DNA Blood System. ACD blood should be used as starting material.

Alternatively, the DNA can be extracted by any preferred method yielding pure DNA. When using alternative methods, the DNA concentration should be adjusted to 30 ng/μl. **Do not use heparinised blood with these methods.**

Recommended DNA concentration using:

EZ1-extracted DNA, 15 ng/μl.

DNA extracted by other methods, 30 ng/μl.

Concentrations exceeding 50 ng/μl will increase the risk for nonspecific amplifications and weak extra bands, especially for HLA Class I high resolution SSP typings. If necessary, dilute the extracted DNA in dH<sub>2</sub>O.

**PCR AMPLIFICATION****101.531-48 – including *Taq* polymerase**

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<sub>2</sub>O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-H<sub>2</sub>O mixture into each of the 2 wells of an HLA-B\*27 typing. **The 8 well PCR plate is marked with the lot number.** Cover the primer tray(s) with the provided adhesive PCR seals. Check that all reaction wells are completely covered to prevent evaporative loss during PCR amplification. The Olerup SSP<sup>®</sup> Compression Pad (Product No. 103.505-06) can be applied on top of the adhesive PCR seals to prevent evaporation during thermal cycling.

**101.531-48u – without *Taq* polymerase**

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<sub>2</sub>O

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Mix well, dispense 10 µl of the DNA-PCR Master Mix-*Taq*-H<sub>2</sub>O mixture into each of the 2 wells of an HLA-B\*27 typing. **The 8 well PCR plate is marked with the lot number.** Cover the primer tray(s) with the provided adhesive PCR seals. Check that all reaction wells are completely covered to prevent evaporative loss during PCR amplification. The *Olerup* SSP® Compression Pad (Product No. 103.505-06) can be applied on top of the adhesive PCR seals to prevent evaporation during thermal cycling.

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
4.	End - hold	RT		if less than 8 hours
		4°C		if longer than 8 hours

Total reaction volume in each well, 10 µl.

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). **Note: Ethidium bromide is a carcinogen. Handle with appropriate personal protective equipment.**

Load the PCR products, preferably using an 8-channel pipette. Load a DNA size marker (100 base pair ladder, DNA Size Marker Product No. 103.202-100 or DNA Size Marker for short gel runs 103.203-100) 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.

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

### PCR MASTER MIX

The PCR Master Mix including with *Taq* polymerase 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 <sub>2</sub> , 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin
glycerol	final concentration of glycerol is 5%
cresol red	final concentration of cresol red is 100 µg/ml

The same PCR Master Mix is used for all *Olerup* SSP kits including *Taq* polymerase.

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 <sub>2</sub> , 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin
glycerol	final concentration of glycerol is 5%
cresol red	final concentration of cresol red is 100 µg/ml

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

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[www.olerup-ssp.com](http://www.olerup-ssp.com)**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	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-B*27 alleles	Other amplified HLA-B alleles <sup>3</sup>
<b>1</b>	145 bp	<b>430 bp</b>	*27:01-27:05:08, 27:05:10-27:11, 27:13-27:15, 27:17, 27:19-27:21, 27:24-27:25, 27:27-27:28, 27:30, 27:32-27:74, 27:76, 27:78-27:84, 27:86-27:91, 27:93-27:100, 27:102-27:105	*44:97
<b>2<sup>4</sup></b>	95 bp	515 bp	*27:01-27:05:15, 27:05:17-27:05:22, 27:08, 27:10, 27:12-27:13, 27:15-27:18, 27:23, 27:25-27:26, 27:28-27:29, 27:31, 27:36-27:40, 27:42, 27:44-27:45, 27:47-27:69, 27:71-27:75, 27:77, 27:79-27:80, 27:82-27:90:02, 27:92-27:101, 27:103-27:105	*37:02, 47:04-47:05, 48:26

<sup>1</sup>Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of HLA-B\*27 SSP typings.

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

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 bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated

<sup>2</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs or a band of 515 base pairs.

Well 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.

<sup>3</sup>Due to the sharing of sequence motifs between HLA-B alleles the B\*44:97 is amplified by primer mix 1 and the B\*37:02, B\*47:04-47:05 and B\*48:26 alleles are amplified by primer mix 2.

<sup>4</sup>HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

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<b>INTERPRETATION TABLE</b>		
<b>HLA-B*27 SSP typing</b>		
<b>Amplification pattern of the B*27:01 to 27:105 alleles<sup>1</sup></b>		
	<b>Well</b>	
	<b>1</b>	<b>2</b>
<b>Length of spec. PCR product</b>	<b>145</b>	<b>95</b>
<b>Length of int. pos. control<sup>2</sup></b>	<b>430</b>	<b>515</b>
<b>5'-primer<sup>3</sup></b>	<b>167</b>	<b>363</b>
	5' -gCT 3'	5' -AAT 3'
<b>3'-primer<sup>4</sup></b>	<b>272</b>	<b>418</b>
	5' -TgC 3'	5' -gTC 3'
<b>Well No.</b>	<b>1</b>	<b>2</b>
<b>HLA-B allele<sup>5</sup></b>		
<b>*27:01-27:05:08, 27:05:10-27:05:15, 27:05:17-27:05:22, 27:08, 27:10, 27:13, 27:15, 27:17, 27:25, 27:28, 27:36-27:40, 27:42, 27:44-27:45, 27:47-27:69, 27:71-27:74, 27:79-27:80, 27:82-27:84, 27:86-27:90:02, 27:93-27:100, 27:103-27:105</b>	<b>1</b>	<b>2</b>
<b>*27:05:09, 27:12, 27:16, 27:18, 27:23, 27:26, 27:29, 27:31, 27:75, 27:77, 27:85, 27:92, 27:101, 37:02, 47:04-47:05, 48:26</b>		<b>2</b>
<b>*27:05:16, 27:06-27:07:03, 27:09, 27:11, 27:14, 27:19-27:21, 27:24, 27:27, 27:30, 27:32-27:35, 27:41, 27:43, 27:46, 27:70, 27:76, 27:78, 27:81, 27:91, 27:102, B*44:97</b>	<b>1</b>	
<b>HLA-B allele<sup>5</sup></b>		
<b>Well No.</b>	<b>1</b>	<b>2</b>

<sup>1</sup>Due to the sharing of sequence motifs between HLA-B alleles the B\*44:97 is amplified by primer mix 1 and the B\*37:02, B\*47:04-47:05 and B\*48:26 alleles are amplified by primer mix 2.

<sup>2</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs or a band of 515 base pairs.

Well 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.

<sup>3</sup>The nucleotide position, in the 2<sup>nd</sup> and 3<sup>rd</sup> exons, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

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<sup>4</sup>The nucleotide position, in the 2<sup>nd</sup> and 3<sup>rd</sup> exons, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>5</sup>The sequence of the B\*270501 allele has been shown to be identical to B\*27:05:02.  
The B\*2722 sequence has been shown to be identical to the corrected B\*27:06 sequence.



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CELL LINE VALIDATION SHEET					
HLA-B*27 unit dose SSP kit					
				Well	
				1	2
				201318101	201318102
				Production No.	
IHWC cell line		HLA-B			
1	9001 SA	*07:02		-	-
2	9280 LK707	*52:01	*73:01	-	-
3	9011 E4181324	*52:01		-	-
4	9275 GU373	*15:10	*53:01	-	-
5	9009 KAS011	*37:01		-	-
6	9353 SM	*39:01	*51:01	-	-
7	9020 QBL	*18:01		-	-
8	9025 DEU	*35:01		-	-
9	9026 YAR	*38:01		-	-
10	9107 LKT3	*54:01		-	-
11	9051 PITOUT	*44:03		-	-
12	9052 DBB	*57:01		-	-
13	9004 JESTHOM	*27:05		+	+
14	9071 OLGA	*15:01	*15:20	-	-
15	9075 DKB	*40:01		-	-
16	9037 SWEIG007	*40:02		-	-
17	9282 CTM3953540	*08:01	*55:01	-	-
18	9257 32367	*14:01	*56:01	-	-
19	9038 BM16	*18:01		-	-
20	9059 SLE005	*40:01		-	-
21	9064 AMALA	*15:01		-	-
22	9056 KOSE	*35:03		-	-
23	9124 IHL	*40:02	*56:02	-	-
24	9035 JBUSH	*38:01		-	-
25	9049 IBW9	*14:02		-	-
26	9285 WT49	*58:01		-	-
27	9191 CH1007	*07:05	*51:01	-	-
28	9320 BEL5GB	*44:02	*44:03	-	-
29	9050 MOU	*44:03		-	-
30	9021 RSH	*42:01		-	-
31	9019 DUCAF	*18:01		-	-
32	9297 HAG	*41:02		-	-
33	9098 MT14B	*40:01		-	-
34	9104 DHIF	*38:01		-	-
35	9302 SSTO	*44:02		-	-
36	9024 KT17	*15:01	*35:01	-	-
37	9065 HHKB	*07:02		-	-
38	9099 LZL	*15:01		-	-
39	9315 CML	*08:01	*27:05	+	+
40	9134 WHONP199	*13:02	*46:01	-	-
41	9055 H0301	*14:02		-	-
42	9066 TAB089	*46:01		-	-
43	9076 T7526	*46:01		-	-
44	9057 TEM	*38:01		-	-
45	9239 SHJO	*42:01	*50:01	-	-
46	9013 SCHU	*07:02		-	-
47	9045 TUBO	*51:01		-	-
48	9303 TER-ND	*35:01	*44:03	-	-

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## CERTIFICATE OF ANALYSIS

### **Olerup SSP<sup>®</sup> HLA-B\*27 SSP – unit dose**

**Product number:** 101.531-48 – including *Taq* polymerase  
101.531-48u – without *Taq* polymerase  
**Lot number:** 65S  
**Expiry date:** 2016-January-01  
**Number of tests:** 48  
**Number of wells per test:** 2

#### **Well specifications:**

Well No.	Production No.
1	2013-181-01
2	2013-181-02

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

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

**Date of approval:** 2013-July-19

**Approved by:**

**Production Quality Control**

101.531-48 – including *Taq* pol., IFU-01  
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## Declaration of Conformity

**Product name:** *Olerup* SSP® HLA-B\*27 - unit dose  
**Product number:** 101.531-48/48u  
**Lot number:** 65S

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

**Manufacturer:** *Olerup* SSP AB  
Franzengatan 5  
SE-112 51 Stockholm, 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 9001:2008 and ISO 13485:2012, 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, Franzengatan 5, SE-112 51 Stockholm, Sweden.

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

Stockholm, Sweden  
2013-July-19

Åsa Olausson  
Production Quality Control

101.531-48 – including **Taq** pol., IFU-01  
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