

101.532-48 – including *Taq* polymerase, IFU-01 Rev. No. 03  
101.532-48u – without *Taq* polymerase, IFU-02 Rev. No. 03

Visit [www.olerup-ssp.com](http://www.olerup-ssp.com) for  
“Instructions for Use” (IFU)

Lot No.: **20N**

Lot-specific information

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

Product number:	101.532-48 – including <i>Taq</i> polymerase 101.532-48u – without <i>Taq</i> polymerase
Lot number:	20N
Expiry date:	2014-February-01
Number of tests:	48
Number of wells per test:	2
Storage - primer vials:	dark at -20°C
- PCR Master Mix:	-20°C
- Control DNAs:	-20°C
- Product Insert	RT

**This Product Description is only valid for Lot No. 20N.**

### **CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*<sup>®</sup> HLA-B\*27 LOT**

The Lot-specific information for HLA- B\*27 including and without *Taq* polymerase is now 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. 83K**) was made.

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

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## 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\*27:86.

The primer solutions consist of specific primer mixes, i.e. 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\*08:01,27:05.**

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.)

**PCR Master Mix complete with Taq**, *Taq* polymerase, nucleotides, buffer, glycerol and cresol red, is included in the kit including *Taq* polymerase.

#### 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 and B\*47:05 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\*27:86**, recognized by the HLA Nomenclature Committee in January 2012<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, 47:04-47:05 and 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 2012-January-12, release 3.7.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

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## 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

#### **101.532-48 – including *Taq* polymerase**

For one HLA-B\*27 bulk typing, dispense 5 μl of each of the 2 HLA-B\*27 bulk primer solutions into an 8 tube strip of 0.2 ml PCR tubes; primer solution 1 into well 1, primer solution 2 into well 2.

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

Mix well, dispense 5 μl of the DNA-PCR Master Mix mixture into each of the 2 wells of an HLA-B\*27 typing. Close the 8 tube PCR strip with an 8 strip lid.

Use a 96 well thermal cycler with a heated lid. The temperature gradient across the heating block should be ≤0.75°C.

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**101.532-48u – without *Taq* polymerase**

For one HLA-B\*27 bulk typing, dispense 5 µl of each of the 2 HLA-B\*27 bulk primer solutions into an 8 tube strip of 0.2 ml PCR tubes; primer solution 1 into well 1, primer solution 2 into well 2.

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 Master Mix without *Taq* – mix well before taking your aliquot

0.3 µl *Taq* polymerase (5 units/µl)

Mix well, dispense 5 µl of the DNA-PCR Master Mix mixture into each of the 2 wells of an HLA-B\*27 typing. Close the 8 tube PCR strip with an 8 strip lid.

Use a 96 well thermal cycler with a heated lid. The temperature gradient across the heating block should be ≤0.75°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), 1 drop of ethidium bromide solution per 50-75 ml of gel, or our GelRed™ dropper bottle (Product No. 103.302-05) 4 drops per 100-120 ml of gel solution. **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, DNA Size Marker Product No. 103.202-100 or DNA Size Marker for short gel runs 103.203-100) in one well per row.

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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**

**101.532-48 – including *Taq* polymerase**

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 <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 complete with *Taq* can be shipped at ambient temperature.

When stored at –20°C, the PCR Master Mix including *Taq* polymerase is stable for 33 months from the date of manufacture.

**101.532-48u – without *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.

The PCR Master Mix without *Taq* can be shipped at ambient temperature.

When stored at –20°C, the PCR Master Mix without *Taq* polymerase is stable for 33 months from the date of manufacture.

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**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>
1	145 bp	430 bp	*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	*44:97
2 <sup>4</sup>	95 bp	515 bp	*27:01-27:05:15, 27:05:17, 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, 27:82-27:86	*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 specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective lengths 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 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, 47:04-47:05, 48:26 alleles are amplified by primer mix 2.

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

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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: 86 alleles</b>		
	<b>Well</b>	
	<b>1</b>	<b>2</b>
<b>Length of spec.</b>	<b>145</b>	<b>95</b>
<b>PCR product</b>		
<b>Length of int. pos. Control<sup>1</sup></b>	<b>430</b>	<b>515</b>
<b>5'-primer<sup>2</sup></b>	<b>167</b>	<b>363</b>
	5' -gCT 3'	5' -AAT 3'
<b>3'-primer<sup>3</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>4,5</sup></b>		
<b>*27:01-27:05:08, 27:05:10-27:05:15, 27:05:17, 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</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, 37:02, 47:04-47:05, 48:26</b>		<b>2</b>
<b>*27:05:16, 27:06-27:07:02, 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, 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>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>2</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.

<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 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>4</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.

<sup>5</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, 47:04-47:05, 48:26 alleles are amplified by primer mix 2.

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<b>CELL LINE VALIDATION SHEET</b>						
<b>HLA-B*27 bulk SSP kit</b>						
				<b>Well</b>		
					<b>1</b>	<b>2</b>
				<b>Production No.</b>	201191201	201191202
<b>IHWC cell line</b>		<b>HLA-B</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® HLA-B\*27 SSP – bulk**

**Product number:** 101.532-48 – including *Taq* polymerase  
101.532-48u – without *Taq* polymerase  
**Lot number:** 20N  
**Expiry date:** 2014-February-01  
**Number of tests:** 48  
**Number of wells per test:** 2

#### **Well specifications:**

Well No.	Production No.
1	2011-912-01
2	2011-912-02

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:** 2012-March-27

**Approved by:**

**Production Quality Control**

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## Declaration of Conformity

**Product name:** *Olerup* SSP® HLA-B\*27 - bulk  
**Product number:** 101.532-48/48u  
**Lot number:** 20N

**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 13485:2003, 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, 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  
2012-March-27

Ann-Cathrin Jareman  
Head of QA and Regulatory Affairs

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