

101.911-96 – including *Taq* pol., IFU-01

101.911-96u – without *Taq* pol., IFU-02

Visit <https://labproducts.caredx.com> for

“Instructions for Use” (IFU)

Lot No.: **0L1**

Lot-specific information

## **Olerup SSP® HLA-B\*27 unit dose single well<sup>1</sup>**

Product number:	101.911-96 – including <i>Taq</i> polymerase 101.911-96u – without <i>Taq</i> polymerase
Lot number:	0L1
Expiry date:	2024-03-01
Number of tests:	96
Number of wells per test:	1
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. 0L1.**

Complete product documentation consists of generic Instructions for Use (IFU), lot specific Product Insert, Worksheet and Certificate.

### **CHANGES COMPARED TO THE PREVIOUS OLERUP SSP® HLA-B\*27 UNIT DOSE SINGLE WELL LOT (9H2)**

The HLA-B\*27 unit dose single well specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP®* HLA-B\*27 lot (**Lot No. 9H2**) was made. The kit design is based on IMGT/HLA database 3.38.0<sup>1</sup>.

The HLA-B\*27 unit dose is unchanged compared to the previous *Olerup SSP®* HLA-B\*27 unit dose single well lot.

1. The primers in this kit do **not** amplify the B\*27:05:23, 27:23, 27:29, 27:75, 27:77, 27:85, 27:129, 27:140, 27:157, 27:189 and 27:204 alleles.

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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\*27:188. The primers in this kit do **not** amplify the B\*27:05:23, 27:23, 27:29, 27:75, 27:77, 27:85, 27:129, 27:140, 27:157, 27:189 and 27:204 alleles.

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.)

We recommend including one positive and one negative control DNA in each test set up. The kit contains enough control DNAs to perform 16 test set ups. If more than 16 test set ups per kit are run other positive and negative DNA samples can be used as controls (e.g. positive and negative samples from previous tests).

#### PLATE LAYOUT

Each 8 well strip is intended for testing 8 sample DNAs.

1	1	1	1	1	1	1	1
---	---	---	---	---	---	---	---

The cut PCR plate is marked with 'B27' in silver/gray ink.

Well No. 1 is marked with the Lot No. '0L1'.

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

Due to the sharing of sequence motifs between HLA-B alleles, non-HLA-B\*27 alleles will be amplified by primer mix 1. For further details see Specificity Table.

The primer mix in this kit does **not** amplify the B\*27:05:23, 27:23, 27:29, 27:75, 27:77, 27:85, 27:129, 27:140, 27:157, 27:189 and 27:204 alleles.

#### UNIQUELY IDENTIFIED ALLELES

All the B\*27 alleles except for the B\*27:05:23, B\*27:23, B\*27:29, B\*27:75, B\*27:77, B\*27:85, B\*27:129, B\*27:140, B\*27:157, B\*27:189 and B\*27:204 alleles, i.e. B\*27:01 to B\*27:221, recognized by the HLA Nomenclature Committee in October 2019<sup>1,2</sup> are identified by the primers in the HLA-B\*27 unit dose single well SSP kit.

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<sup>1</sup>HLA-B alleles listed on the IMGT/HLA web page 2019-October-17, release 3.38.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

<sup>2</sup>Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page <http://hla.alleles.org/alleles/deleted.html>.

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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 A<sub>260/280</sub> 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

#### **101.911-96 – including *Taq* polymerase**

For one HLA-B\*27 typing add at room temperature to a PCR well:

2 μl sample or control DNA (30 ng/μl)

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

5 μl dH<sub>2</sub>O

#### **101.911-96u – without *Taq* polymerase**

For one HLA-B\*27 typing add at room temperature to a PCR well:

2 μl sample or control DNA (30 ng/μl)

3 μl PCR Master Mix without *Taq* polymerase – mix well before taking your aliquot

0.1 μl *Taq* polymerase (5 units/μl)

4.9 μl dH<sub>2</sub>O

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**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.

**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.

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

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### Expected results:

Dependent on the presence or absence and the specificity of the B\*27 alleles in the sample the following results are possible (B\*27 alleles not amplified by the kit and co-amplified non-B27 alleles are excluded):

Mix	Result
+	B*27 positive
-	B*27 negative

Positive control: The positive control mix has to be positive for the B\*27 specific product as defined in the lot-specific Interpretation and Specificity Tables. Absence of B\*27 specific bands in one or both wells might indicate failure of the test.

The negative control DNA must only give rise to the internal control bands of 430 or 515 base pairs respectively and no B\*27 specific bands. Additional bands might indicate inappropriate test conditions or contamination.

### PCR MASTER MIX

The PCR Master Mix including *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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## SPECIFICITY TABLE

### HLA-B\*27 SSP typing

Specificity and size of the PCR product of the HLA-B\*27 unit dose single well SSP typing.

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-B*27 alleles <sup>3</sup>	Other amplified HLA-B alleles
1	145 bp, 205 bp	430 bp	27:01-27:05:22, 27:05:24-27:21, 27:24-27:28, 27:30- 27:74, 27:76, 27:78-27:84, 27:86- 27:128, 27:130- 27:139, 27:141- 27:156, 27:158- 27:188, 27:190- 27:203, 27:205- 27:221	*07:197, 15:342 <sup>w</sup> , 15:395, 38:22, 40:75, 40:322, 44:97, 44:263, 49:26

<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 alleles listed are specified according to amplicon length.

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 control primer pair gives rise to a band of 430 base pairs.

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

<sup>3</sup>The primers in this kit do not amplify the B\*27:05:23, 27:23, 27:29, 27:75, 27:77, 27:85, 27:129, 27:140, 27:157, 27:189 and 27:204 alleles.

#### Abbreviations

w: may be weakly amplified.

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## PRIMER SPECIFICATION

Well No.	1
Length of spec.	145
PCR product	205
Length of int.	430
pos. control <sup>1</sup>	
5'-primer(s) <sup>2</sup>	167
	5' -gCT 3'
	363
	5' -AAT 3'
3'-primer(s) <sup>3</sup>	272
	5' -TgC 3'
	272
	5' -TgC 3'
	527
	5' -CCT 3'
Well No.	1

<sup>1</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The control primer pair gives rise to a band of 430 base pairs.

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

<sup>2</sup>The nucleotide position 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 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.



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CELL LINE VALIDATION SHEET				
HLA-B*27 unit dose SSP kit <sup>2</sup>				
				Well
				1
			Production No.	202018001
	IHWC cell line <sup>1</sup>	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 S8TO	*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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<sup>1</sup>The provided cell line HLA specificities are retrieved from the <http://www.ihwg.org/hla> web site. The specificity of an individual cell line may thus be subject to change.

<sup>2</sup>The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs.

One 3'-primer was tested by separately adding one 5'-primer.

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