101.531-48 – including *Taq* polymerase, IFU-01
101.531-48u – without *Taq* polymerase, IFU-02

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Lot No.: 3H1 **Lot-specific information**

Olerup SSP® HLA-B*27 – unit dose

Product number:	**101.531-48 – including *Taq* polymerase** **101.531-48u – without *Taq* polymerase**
Lot number:	**3H1**
Expiry date:	**2021-07-01**
Number of tests:	**48**
Number of wells per test:	**2**
Storage - pre-aliquoted primers:	**dark at -20ºC**
- PCR Master Mix:	**-20ºC**
- Control DNAs:	**-20ºC**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 3H1.

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 LOT (3G2)

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. 3G2)** was made. The kit design is based on IMGT/HLA database 3.34.0.

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

Lot No.: 3H1 **Lot-specific information**

PRODUCT DESCRIPTION

HLA-B*27 SSP typing

CONTENT

The primer set contains two vials of 5'- and 3'-primers for identifying the HLA-B27 specificity, B*27:01 to B*27:182.
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.)

We recommend including one positive and one negative control DNA in each test set up. The kit contains enough control DNAs to perform 8 test set ups. If more than 8 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).

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

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 'B27' in silver/gray ink.
Well No. 1 is marked with the Lot No. '3H1'.
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, a few non-HLA-B*27 alleles will be amplified by primer mixes 1 and 2. For further details see Specificity Table.

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UNIQUELY IDENTIFIED ALLELES

All the HLA-B*27 alleles, i.e. **B*27:01 to B*27:182**, recognized by the HLA Nomenclature Committee in October 2018[1,2] are identified by the primers in the HLA-B*27 SSP kit.

[1]HLA-B*27 alleles listed on the IMGT/HLA web page 2018-October-18, release 3.34.0, www.ebi.ac.uk/imgt/hla.
[2]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 dH2O. 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 dH2O.

PCR AMPLIFICATION

101.531-48 – including Taq polymerase
Per sample, add at room temperature in a 0.5 ml tube:
3 x 2 µl = 6 µl DNA (30 ng/µl)
3 x 3 µl = 9 µl PCR Master Mix complete with *Taq* – mix well before taking your aliquot
3 x 5 µl = 15 µl dH_2O

Mix well, dispense 10 µl of the DNA-PCR Master Mix 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 ≤0.75°C.

101.531-48u – without Taq polymerase
Prepare a PCR Master Mix with Taq in a 0.5 ml tube. Per well add:
3 µl of Master Mix and 0.075 µl of Taq (5 units/µl).

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Calculate the amount of PCR Master Mix and Taq required by multiplying the above volumes with the number of wells needed for all samples and the controls. Take care to prepare a higher volume than actually needed in order to account for pipetting losses etc. or use the table below for recommended volumes.

Table 1. Mastermix preparation for HLA-B*27 unit dose.

No. of wells per set up	Volume of Master Mix (µl)	Volume of *Taq* polymerase (µl)
6	24	0.6
8	30	0.8
10	36	1.0
12	42	1.1
14	48	1.3
16	54	1.4
18	63	1.7
20	69	1.8
22	75	2.0
24	81	2.2
26	90	2.4
28	96	2.6
30	102	2.7
32	108	2.9
36	126	3.4
44	150	4.0
48	162	4.3
56	186	5.0
64	210	5.6
72	240	6.4
80	264	7.0
88	288	7.7
96	312	8.3

Per sample, add at room temperature in a 0.5 ml tube:
3 x 2 µl = 6 µl DNA (30 ng/µl)
3 x 3 µl = 9 µl Master Mix with Taq prepared in the previous step – mix well before taking your aliquot
3 x 5 µl = 15 µl dH_2O
Mix well, dispense 10 µl of the DNA-PCR Master Mix 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 < 0.75°C.

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PCR cycling parameters:

1.	1 cycle	94ºC	2 min	denaturation
2.	10 cycles	94ºC	10 sec.	denaturation
		65ºC	60 sec.	annealing and extension
3.	20 cycles	94ºC	10 sec.	denaturation
		61ºC	50 sec.	annealing
		72ºC	30 sec.	extension
4.	End - hold	RT		if less than 8 hours
		4ºC		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 60ºC. 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 GelRedTM 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.

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***.

Lot No.: 3H1 **Lot-specific information**

Expected results:

Dependent on the presence or absence and the specificity of the B*27 alleles in the sample the following results are possible (the co-amplified non-B27 alleles excluded):

Table 2. Interpretation of results.

Mix1	Mix2	Result
+	-	B*27 positive
-	+	B*27 positive
+	+	B*27 positive
-	-	B*27 negative

Positive control: Both mixes have to be positive for the B*27 specific products 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 with *Taq* polymerase 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 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 $MgCl_2$, 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 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 two primer mixes used for HLA-B*27 SSP typing.

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified HLA-B*27 alleles	Other amplified HLA-B alleles
1	145 bp	**430 bp**	*27:01-27:05:08, 27:05:10-27:05:22, 27:05:24-27:17, 27:19-27:21, 27:24-27:25, 27:27-27:28, 27:30, 27:32-27:74, 27:76, 27:78-27:84, 27:86-27:100, 27:102-27:128, 27:130-27:139, 27:141-27:156, 27:158-27:182	*38:22, 40:75, 40:322, 44:97, 44:263, 49:26
2[3]	95 bp	515 bp	*27:01-27:05:15, 27:05:17-27:05:36, 27:08, 27:10, 27:12:01:01-27:13, 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, 27:82-27:90:04, 27:92-27:101, 27:103-27:105, 27:108-27:124, 27:126, 27:128-27:129, 27:131-27:135, 27:137, 27:139-27:149, 27:151-27:152, 27:155-27:163, 27:165-27:167, 27:169-27:179, 27:181-27:182	*07:197, 15:342, 37:02, 47:04-47:05, 48:26, **A*32:89**

[1]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 inherit 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.
[2]The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 430 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.
[3]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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PRIMER SPECIFICATION

Well No.	1	2
Length of spec.	145	95
PCR product		
Length of int.	430	515
pos. control[1]		
5'-primer(s)[2]	167	363
	5' -gCT 3'	5' -AAT 3'
3'-primer(s)[3]	272	418
	5' -TgC 3'	5' -gTC 3'
	272	
	5' -TgC 3'	
Well No.	1	2

[1]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.

[2]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 web site. The sequence of the 3 terminal nucleotides of the primer is given.

[3]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 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[2]

					Well	
					1	2
				Production No.	201901101	201901102
	IHWC cell line[1]		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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[1]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.

[2]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 additional 3'-primer in primer mix 1 was tested by separately adding one additional 5'-primer.

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