

Olerup SSP[®] 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:	49E
Expiry date:	2010-May-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. 49E.

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. Y02**) was made.

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

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*2742.

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.)

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
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The 8 well cut PCR plate is marked with 'HLA-B*27'.

Well No. 1 is marked with the Lot No. '49E'.

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 HLA-B*27 alleles, i.e. **B*2701 to B*2742**, recognized by the HLA Nomenclature Committee in April 2008¹ 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.

¹HLA-B alleles listed on the IMGT/HLA web page 2008-April-08, release 2.21.0, www.ebi.ac.uk/imgt/hla.

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 ³
1	145 bp	430 bp	2701-270402, 270502-270508, 270510, 2706-2711, 2713-2715, 2717, 2719-2721, 2724, 2725, 2727, 2728, 2730, 2732-2742	
2 ⁴	95 bp	515 bp	2701-270402, 270502-270510, 2708, 2710, 2712, 2713, 2715-2718, 2723, 2725, 2726, 2728, 2729, 2731, 2736-2740, 2742	3702, 4704, 4705

¹Alleles 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 length 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

²The 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.

³Due to the sharing of sequence motifs between HLA-B alleles three non-HLA-B*27 alleles will be amplified by primer mix 2.

⁴Short 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 2742 alleles¹			
	Well		
	1	2	
Length of spec.	145	95	Length of spec.
PCR product			PCR product
Length of int.	430	515	Length of int.
pos. control²			pos. control
5'-primer³	167	363	5'-primer³
	5'-gCT ^{3'}	5'-AAT ^{3'}	
3'-primer⁴	272	418	3'-primer⁴
	5'-TgC ^{3'}	5'-gTC ^{3'}	
Well No.	1	2	Well No.
HLA-B allele⁵			HLA-B allele⁵
*2701-270402, 270502-270508, 270510, 2708, 2710, 2713, 2715, 2717, 2725, 2728, 2736-2740, 2742	1	2	*2701-270402, 270502-270508, 270510, 2708, 2710, 2713, 2715, 2717, 2725, 2728, 2736-2740, 2742
*2706, 2707, 2709, 2711, 2714, 2719-2721, 2724, 2727, 2730, 2732-2735, 2741	1		*2706, 2707, 2709, 2711, 2714, 2719-2721, 2724, 2727, 2730, 2732-2735, 2741
*270509, 2712, 2716, 2718, 2723, 2726, 2729, 2731, 3702, 4704, 4705		2	*270509, 2712, 2716, 2718, 2723, 2726, 2729, 2731, 3702, 4704, 4705
HLA-B allele⁵			HLA-B allele⁵
Well No.	1	2	Well No.

¹Due 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.

²The 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.

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

⁴The 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 on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

⁵The 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					
			Well		
				1	2
			Production No.	200845301	200 845 302
	IHWC 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 – including *Taq* polymerase
101.531-48u – without *Taq* polymerase
Lot number: 49E
Expiry date: 2010-May-01
Number of tests: 48
Number of wells per test: 2

Well specifications:

Well No.	Production No.
1	2008-453-01
2	2008-453-02

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: 2008-May-27

Approved by:

Quality Control, Supervisor

Lot No.: **49E**

Lot-specific information

www.olerup.com

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*27 - unit dose
Product number: 101.531-48/101.531-48U
Lot number: 49E

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

Manufacturer: *Olerup* SSP AB
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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.

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Saltsjöbaden, Sweden
2008-May-27

Olle Olerup
Managing Director

HLA-B*27 – unit dose
101.531-48 – including *Taq* polymerase
101.531-48u – without *Taq* polymerase

Product Insert

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Lot No.: **49E**

Lot-specific information

www.olerup.com

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