Olerup SSP® HLA-B*82

Product number:	**101.552-06 – including *Taq* polymerase**
Lot number:	**27K**
Expiry date:	**2012-July-01**
Number of tests:	**6**
Number of wells per test:	**5**
Storage - pre-aliquoted primers:	**dark at -20°C**
- PCR Master Mix:	**-20°C**
- Adhesive PCR seals	**RT**
- Product Insert	**RT**

This Product Description is only valid for Lot No. 27K.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® HLA-B*82 LOT.

The HLA-B*82 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup* SSP® HLA-B*82 lot was made **(Lot No. 57F)**.

One well has been added to the B*82 kit,
well **5**.

The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot.

Well	5'-primer	3'-primer	rationale
2	-	-	Exchanged positive control primer pair.
5	New	New	New primer pair for the B*82:03 allele.

PRODUCT DESCRIPTION

HLA-B*82 SSP typing

CONTENT
The primer set contains 5'- and 3'-primers for identifying the B*82:01 to B*82:03 alleles.

PLATE LAYOUT
Each HLA-B*82 test consists of 5 PCR reactions in an 8 well cut PCR plate. Wells 6 to 8 are empty.

1	**2**	**3**	**4**	**5**	**empty**	**empty**	**empty**

The 8 well PCR plate is marked with 'B82' in silver/gray ink.
Well No. 1 is marked with the Lot No. '27K'.
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 heat-sealed with a PCR-compatible foil.
Please note: When removing each 8 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION
The interpretation of HLA-B*82 SSP subtypings will be influenced by six B*15, three B*44, five B*45 and the B*50:02 alleles when present on the other haplotype. In addition, the C*04:08 and C*04:34 alleles will be amplified by primer mix 3.

UNIQUELY IDENTIFIED ALLELES
All the HLA-B*82, i.e. **B*82:01 to B*82:03**, recognized by the HLA Nomenclature Committee in April 2010[1] will be amplified by the primers in the HLA-B*82 SSP kit.

[1]HLA-B alleles listed on the IMGT/HLA web page 2010-April-01, release 3.0.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES
The three HLA-B*82 alleles can be combined in 6 homozygous and heterozygous combinations. Two of these genotypes do not give rise to unique amplification patterns.

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++-++      82:01,82:03 = 82:03,82:03
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SPECIFICITY TABLE

HLA-B*82 SSP subtyping

Specificities and sizes of the PCR products of the 5 primer mixes used for HLA-B*82 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified HLA-B*82 alleles	Other amplified HLA Class I alleles[3]
1	195 bp	**800 bp**	*82:01-82:03	*44:10, 44:15, 44:18, 45:01, 45:05-45:07, 45:11, 50:02
2	140 bp	**800 bp**	*82:01, 82:03	
3	230 bp	1070 bp	*82:02	*15:06, 15:27:01-15:27:03, 15:84, 15:109, **C*04:08, C*04:34**
4	195 bp	1070 bp	*82:01-82:03	
5	155 bp	1070 bp	*82:03	

[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*82 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 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 two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.
Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*82 SSP subtyping. In addition, well number 2 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.
In the presence of a specific amplification the intensity of the control band often decreases.
[3]Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B*82 alleles will be amplified by primer mixes 1 and 3. In addition, the C*04:08 and C*04:34 alleles will be amplified by primer mix 3.

INTERPRETATION TABLE

HLA-B*82 SSP subtyping

Amplification patterns of the B*82 alleles

	Well				
	1	**2**	**3**	**4**	**5**
Length of spec. PCR product	**195**	**140**	**230**	**195**	**155**
Length of int. pos. control[1]	**800**	**800**	**1070**	**1070**	**1070**
5'-primer[2]	**420**	**557**	**368**	**105**	**105**
	5' -TTA 3'	5' -ggA 3'	5' -gTT 3'	5' -gCT 3'	5' -gCT 3'
3'-primer[3]	**572**	**3rd I**	**557**	**259**	**219**
	5' -gCg 3'	5' -TAT 3'	5' -ggC 3'	5' -gTT 3'	5' -ggg 3'
				259	
				5' -gTT 3'	
Well No.	**1**	**2**	**3**	**4**	**5**
HLA-B allele					
***82:01**	**1**	**2**		**4**	
***82:02**	**1**		**3**	**4**	
***82:03**	**1**	**2**		**4**	**5**
15:06, 15:27:01-15:27:03, 15:84, 15:109, *C*04:08, C*04:34			**3**		
***44:10, 44:15, 44:18, 45:01, 45:05-45:07, 45:11, 50:02**	**1**				
HLA-B allele					
Well No.	**1**	**2**	**3**	**4**	**5**

[1]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 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.
Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*82 SSP subtyping. In addition, well number 2 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.
In the presence of a specific amplification the intensity of the control band often decreases.
[2]The nucleotide position, in the 2nd or 3rd exon, 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, in the 2nd or 3rd exon or 3rd intron 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.

CELL LINE VALIDATION SHEET

HLA-B*82 SSP primer set

					Well				
					1	2	3	4	5
				Prod. No.:	200956201	201074202	200956203	200956204	201074205
	IHWC cell line		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	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-B*82 SSP

Product number: **101.552-06 – including *Taq* polymerase**
Lot number: **27K**
Expiry date: **2012-July-01**
Number of tests: **6**
Number of wells per test: **5**

Well specifications:

Well No.	Production No.
1	2009-562-01
2	2010-742-02
3	2009-562-03
4	2009-562-04
5	2010-742-05

The specificity of each primer solution of the HLA-B*82 primer set has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 3 and 5 were available. The specificity of the primers in primer solution 3 was tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 5, it was only possible to test the 5'-primer, the 3'-primer was not possible to test.
In addition, one of the 3'-primers in primer solution 4 was tested by adding an additional 5'-primer.

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

Date of approval: 2010-July-16

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*82
Product number: 101.552-06
Lot number: 27K

Intended use: HLA-B*82 high resolution histocompatibility testing

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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 9001:2008 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, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

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

Saltsjöbaden, Sweden
2010-July-16

Olle Olerup
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