

Lot No.: **56F**

Lot-specific information

***Olerup SSP*[®] HLA-B*81**

Product number: 101.553-06u – without *Taq* polymerase
Lot number: 56F
Expiry date: 2011-February-01
Number of tests: 6
Number of wells per test: 6
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. 56F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] HLA-B*81 LOT.

The HLA-B*81 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP*[®] HLA-B*81 lot was made (Lot No. 18E).

One well has been added to the HLA-B*81 kit,
well **6**.

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

Well	5'-primer	3'-primer	rationale
3	-	Exchanged	New 3'-primer to increase specificity of amplification.
5	Exchanged	-	New 5'-primer to improve resolution of the B*8101 and B*8103 alleles.
6	New	New	New primer pair for the B*8104N allele.

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PRODUCT DESCRIPTION

HLA-B*81 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for separating the B*8101 to B*8104N alleles.

PLATE LAYOUT

Each HLA-B*81 test consists of 6 PCR reactions in an 8 well cut PCR plate. Wells 7 and 8 are empty.

1	2	3	4	5	6	empty	empty
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The 8 well PCR plate is marked with 'B81' in silver/gray ink.

Well No. 1 is marked with the Lot No. '56F'.

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*81 SSP subtypings will be influenced by most B*07, most B*08, the B*1520, most B*27, most B*35, the B*3707, six B*40, two B*41, the B*42, the B*4506, the B*48, the B*51, the B*52, the B*53, the B*54, the B*55, the B*56, the B*58, the B*6701, the B*78, the B*82 and the B*8301 alleles when present on the other haplotype.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*81 alleles, i.e. **B*8101 to B*8104N**, recognized by the HLA Nomenclature Committee in January 2009¹ will be amplified by the primers in the HLA-B*81 SSP kit.

¹HLA-B alleles listed on the IMGT/HLA web page 2009-January-16, release 2.24.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 4 HLA-B*81 alleles can be combined in 10 homozygous and heterozygous combinations. Six of these genotypes do not give rise to unique amplification patterns.

+++++ 8101,8103 = 8102,8103 = 8103,8103
+++++ 8101,8104N = 8102,8104N = 8104N,8104N

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SPECIFICITY TABLE

HLA-B*81 SSP subtyping

Specificities and sizes of the PCR products of the 6 primer mixes used for HLA-B*81 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*81 alleles	Other amplified HLA-B alleles ³
1	175 bp	800 bp	8101-8104N	2724, 4031, 4045, 4080, 480101-480102, 4804, 4806, 4807, 4809, 4811, 4815, 4816, 4818, 4819
2	410 bp	1070 bp	8101, 8103 [?] , 8104N [?]	2702-270402, 270502-2711, 2713-2715, 2717, 2719-2721, 2724-2728, 2730-2738, 2740-2745, 3576, 5401-5417, 550101-5505, 5507-5517, 5519-5534, 5601-5616, 5618-5622, 5624, 8201, 8202, 8301
3 ⁴	410 bp	1070 bp	8102, 8103 [?] , 8104N [?]	070201-070207, 0704-0707, 0709- 0715, 0717-0726, 0728-0731, 0733-0736, 0739-0746, 0748, 0749N, 0751-0764, 0766-0768, 4201, 4202, 4204-4206, 4208, 4209, 4506, 670101-6702, 7301
4	285 bp	1070 bp	8101-8104N [?]	070201-0706, 0708-0711, 0713, 0715-0717, 0720-0732, 0734-0739, 0741-0752, 0754-0759, 0761-0768, 3566, 3707, 4015, 4016, 4031, 4032, 4045, 4080, 420501-420502, 480101-480102, 4805-4812, 4814-4816, 4818, 4819
5	165 bp	1070 bp	8103	350101-3530, 3532-

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				3542, 3544, 3545, 3547-3556, 3558, 3559, 3561-3572, 3574-3578, 3580-3586, 3588-3594, 4802, 510101-512402, 5126- 5141N, 5143-5146, 5148-5163, 520101- 520602, 5208-5214, 530101-5316, 580101- 5802, 5804-5810N, 5812-5819, 7801-7806
6	80 bp	1070 bp	8104N	

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*81 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 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 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 non-HLA-B*81 alleles will be amplified by primer mixes 1 to 5.

⁴Primer mix 3 may give rise to a primer oligomer artifact.

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INTERPRETATION TABLE						
HLA-B*81 SSP subtyping						
Amplification patterns of the B*81 alleles						
	Well					
	1	2	3	4	5	6
Length of spec.	175	410	410	285	165	80
PCR product						
Length of int.	800	1070	1070	1070	1070	1070
pos. control¹						
5'-primer²	363	41	44	363	652	206
	<small>⁵-AgC³</small>	<small>⁵-CTg³</small>	<small>⁵-ggC³</small>	<small>⁵-AgC³</small>	<small>⁵-CCg³</small>	<small>⁵-AgA³</small>
3'-primer³	499	282	282	605	774	244
	<small>⁵-ggA³</small>	<small>⁵-gCC³</small>	<small>⁵-gCC³</small>	<small>⁵-gCT³</small>	<small>⁵-ggT³</small>	<small>⁵-CTA³</small>
Well No.	1	2	3	4	5	6
HLA-B allele						
*8101	1	2		4		
*8102	1		3	4		
*8103	1	?	?	4	5	
*8104N	1	?	?	4		6
*070201-070207, 0704-0706, 0709-0711, 0713, 0715, 0717, 0720-0726, 0728-0731, 0734-0736, 0739, 0741-0746, 0748, 0749N, 0751, 0752, 0754-0759, 0761-0764, 0766-0768, 420501-420502			3	4		
*0703, 0708, 0716, 0727, 0732, 0737, 0738, 0747, 0750, 0765, 3707, 4015, 4016, 4032, 4805, 4808, 4810, 4812, 4814				4		
*0707, 0712, 0714, 071801-0719, 0733, 0740, 0753, 0760, 4201, 4202, 4204, 4206, 4208, 4209, 4506, 670101-6702, 7301			3			
*2702-270402, 270502-2711, 2713-2715, 2717, 2719-2721, 2725-2728, 2730-2738, 2740-2745, 5401-5417, 550101-5505, 5507-5517, 5519-5534, 5601-5616, 5618-5622, 5624, 8201, 8202, 8301		2				
Well No.	1	2	3	4	5	6

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Length of spec.	175	410	410	285	165	80
PCR product						
Well No.	1	2	3	4	5	6
*2724	1	2				
*350101-3530, 3532-3542, 3544, 3545, 3547-3556, 3558, 3559, 3561-3565Q, 3567-3572, 3574, 3575, 3577, 3578, 3580-3586, 3588-3594, 510101-512402, 5126-5141N, 5143-5146, 5148-5163, 520101-520602, 5208-5214, 530101-5316, 580101-5802, 5804-5810N, 5812-5819, 7801-7806					5	
*3566				4	5	
*3576		2			5	
*4031, 4045, 4080, 480101-480102, 4806, 4807, 4809, 4811, 4815, 4816, 4818, 4819	1			4		
*4804	1					
HLA-B allele						
Well No.	1	2	3	4	5	6

¹The 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.

²The nucleotide position, in the 1st, 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.

³The nucleotide position, in the 2nd or 3rd exon, 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*81 SSP primer set										
					Well					
					1	2	3	4	5	6
				Prod. No.:	200956101	200956102	200956103	200956104	200956105	200956106
IHC cell line			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	9004	JESTHOM	*2705		-	+	-	-	-	-
14	9071	OLGA	*1501	*1520	-	-	-	-	+	-
15	9075	DKB	*4001		-	-	-	-	-	-
16	9037	SWEIG007	*4002		-	-	-	-	-	-
17	9282	CTM3953540	*0801	*5501	-	+	-	-	-	-
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	-	-	-	-	+	-

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CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-B*81 SSP

Product number: 101.553-06u – without *Taq* polymerase
Lot number: 56F
Expiry date: 2011-February-01
Number of tests: 6
Number of wells per test: 6

Well specifications:

Well No.	Production No.
1	2009-561-01
2	2009-561-02
3	2009-561-03
4	2009-561-04
5	2009-561-05
6	2009-561-06

The specificity of each primer solution of the HLA-B*81 primer set has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solution 6 were available. The specificities of the primers in this primer solution was tested by separately adding one additional 3'-primer, the 3'-primer was not possible to test.

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

Date of approval: 2009-May-27

Approved by:

Quality Control, Supervisor

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Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*81
Product number: 101.553-06u
Lot number: 56F

Intended use: HLA-B*81 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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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 9001:2000 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.

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
2009-May-27

Olle Olerup
Managing Director

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