

## Olerup SSP<sup>®</sup> HLA-B\*81

Product number:	101.553-06 – including Taq polymerase 101.553-06u – without Taq polymerase
Lot number:	18E
Expiry date:	2010-March-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. 18E.**

### CHANGES COMPARED TO THE PREVIOUS OLERUP SSP<sup>®</sup> 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<sup>®</sup> HLA-B\*81 lot was made (Lot No. X11).

One well has been added to the HLA-B\*81 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
5	New	New	New primer pair for the B*8103 allele.

Changes in revision R01 compared to R00:

1. Alleles amplified by primer mix 5 has been corrected in the Specificity Table and the Interpretation Table.

## PRODUCT DESCRIPTION

### HLA-B\*81 SSP typing

#### CONTENT

The primer set contains 5'- and 3'-primers for separating the B\*8101 and B\*8103 alleles.

#### PLATE LAYOUT

Each HLA-B\*81 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
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The 8 well PCR plate is marked with 'B\*81'.

Well No. 1 is marked with the Lot No. '18E'.

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\*8103**, recognized by the HLA Nomenclature Committee in January 2008<sup>1</sup> will be amplified by the primers in the HLA-B\*81 SSP kit.

<sup>1</sup>HLA-B alleles listed on the IMGT/HLA web page 2008-January-11, release 2.20.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

#### RESOLUTION IN HOMO- AND HETEROZYGOTES

The 3 HLA-B\*81 alleles can be combined in 6 homozygous and heterozygous combinations. All but two of these genotypes give rise to unique amplification patterns.

++++      8101,8101 = 8101,8103

## SPECIFICITY TABLE

### HLA-B\*81 SSP subtyping

Specificities and sizes of the PCR products of the 5 primer mixes used for HLA-B\*81 SSP subtyping

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-B*81 alleles	Other amplified HLA-B alleles <sup>3</sup>
1	175 bp	800 bp	8101-8103	2724, 4031, 4045, 4080, 480101-480102, 4804, 4806, 4807, 4809, 4811, 4815, 4816, 4818, 4819
2	410 bp	1070 bp	8101, 8103 <sup>?</sup>	2702-270402, 270502-2711, 2713-2715, 2717, 2719-2721, 2724-2728, 2730-2738, 3576, 5401-5413, 550101-5505, 5507-5517, 5519-5529, 5601-5616, 5618-5621, 8201, 8202, 8301
3	400 bp	1070 bp	8102, 8103 <sup>?</sup>	070201-070207, 0704-0707, 0709, 0711, 0712, 0714, 0715, 0717-0726, 0728-0731, 0733-0736, 0739-0749N, 0751-0762, 4201, 4202, 4204-4206, 4208, 4209, 4506, 670101-670102
4	285 bp	1070 bp	8101-8103	070201-0706, 0708-0711, 0713, 0715-0717, 0719-0732, 0734-0741, 0743-0752, 0754-0759, 0761, 0762, 080101-0808N, 0810, 0811, 0813-0815, 0817-0830N, 0832-0835, 3566, 3707, 4015, 4016,

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				4031, 4032, 4045, 4080, 4102, 4104, 4201, 4202, 420501-4207, 4209, 480101-480102, 4805-4812, 4814- 4816, 4818, 4819
<b>5<sup>4</sup></b>	165 bp	1070 bp	8101, 8102	070201-0757, 0759- 0762, 080101-0835, 1301-1304, 1306- 1321, 1401-1407N, 15010101-1519, 1521, 1523-1540, 1542-1558, 1560- 1599, 9501, 9503- 9529, 9531-9539, 180101-1815, 1817N-1828, 2701- 270402, 270502- 2721, 2723-2739, 3531, 3543, 3546, 3557, 3560, 3579, 370101-3713, 380101-3817, 39010101- 39010102L, 390103-3920, 3922- 3943, 400101-4016, 4018-4040, 4042- 4080, 4101-4108, 4201, 4202, 4204- 4209, 44020101- 4459, 4501-4507, 460101-4612, 47010101-4705, 480101-480102, 480301-4819, 4901- 4905, 5001, 5002, 5004, 5207, 5401- 5413, 550101- 550104-5505, 5507- 5529, 5601-5620, 570101-5716, 5811, 5901, 5902, 670101-6702, 8201, 8202, 8301

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<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\*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.

<sup>2</sup>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 the presence of a specific amplification the intensity of the control band often decreases.

<sup>3</sup>Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B\*81 alleles will be amplified by primer mixes 1 to 5.

<sup>4</sup>Primer mix 5 may give rise a primer oligomer artefact. For many HLA-B alleles fourth exon nucleotide sequences are not available. We assume that unknown sequences in the fourth exon are conserved within allelic groups.

<b>INTERPRETATION TABLE</b>					
<b>HLA-B*81 SSP subtyping</b>					
<b>Amplification patterns of the B*81 alleles</b>					
	<b>Well</b>				
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
<b>Length of spec.</b>	<b>175</b>	<b>410</b>	<b>400</b>	<b>285</b>	<b>165</b>
<b>PCR product</b>					
<b>Length of int.</b>	<b>800</b>	<b>1070</b>	<b>1070</b>	<b>1070</b>	<b>1070</b>
<b>pos. control<sup>1</sup></b>					
<b>5'-primer<sup>2</sup></b>	<b>363</b>	<b>44</b>	<b>44</b>	<b>363</b>	<b>652</b>
	5'-AgC <sup>3'</sup>	5'-CTg <sup>3'</sup>	5'-ggC <sup>3'</sup>	5'-AgC <sup>3'</sup>	5'-CCA <sup>3'</sup>
<b>3'-primer<sup>3</sup></b>	<b>499</b>	<b>282</b>	<b>272</b>	<b>605</b>	<b>774</b>
	5'-ggA <sup>3'</sup>	5'-gCC <sup>3'</sup>	5'-TgT <sup>3'</sup>	5'-gCT <sup>3'</sup>	5'-ggT <sup>3'</sup>
<b>Well No.</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
<b>HLA-B allele</b>					
<b>*8101</b>	<b>1</b>	<b>2</b>		<b>4</b>	<b>5</b>
<b>*8102</b>	<b>1</b>		<b>3</b>	<b>4</b>	<b>5</b>
<b>*8103</b>	<b>1</b>	<b>?</b>	<b>?</b>	<b>4</b>	
<b>*070201-070207, 0704-0706, 0709, 0711, 0715, 0717, 0719-0726, 0728-0731, 0734-0736, 0739-0741, 0743-0749N, 0751, 0752, 0754-0759, 0761, 0762, 4201, 4202, 420501-4206, 4209</b>			<b>3</b>	<b>4</b>	<b>5</b>
<b>*0703, 0708, 0710, 0713, 0716, 0727, 0732, 0737, 0738, 0750, 080101-0808N, 0810, 0811, 0813-0815, 0817-0830N, 0832-0835, 3707, 4015, 4016, 4032, 4102, 4104, 4207, 4805, 4808, 4810, 4812, 4814</b>				<b>4</b>	<b>5</b>
<b>*0707, 0712, 0714, 071801-071802, 0733, 0742, 0753, 0760, 4204, 4208, 4506, 670101-670102</b>			<b>3</b>		<b>5</b>
<b>Well No.</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>

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Lot-specific information

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Length of spec.	175	410	400	285	165
PCR product					
Well No.	1	2	3	4	5
*0809, 0812, 0816, 0831, 1301-1304, 1306-1321, 1401-1407N, 15010101-1519, 1521, 1523-1540, 1542-1558, 1560-1599, 9501, 9503-9529, 9531-9530, 180101-1815, 1817N-1828, 2712, 2716, 2718, 2723, 2729, 2739, 3531, 3543, 3546, 3557, 3560, 3579, 370101-3706, 3708-3713, 380101-3817, 39010101-39010102L, 390103-3920, 3922-3943, 400101-401403, 4018-4030, 4033-4040, 4042-4044, 4046-4079, 4101, 410301-410302, 4105-4108, 44020101-4459, 4501-4505, 4507, 460101-4612, 47010101-4705, 480301-480302, 4813, 4817, 4901-4905, 5001, 5002, 5004, 5207, 5518, 5617, 570101-5716, 5811, 5901, 5902, 6702					5
2702-270402, 270502-2711, 2713-2715, 2717, 2719-2721, 2725-2728, 2730-2738, 5401-5413, 550101-5505, 5507-5517, 5519-5529, 5601-5616, 5618-5620, 8201, 8202, 8301		2			5
*2724	1	2			5
*3566				4	
*3576, 5621		2			
*4031, 4045, 4080, 480101-480102, 4806, 4807, 4809, 4811, 4815, 4816, 4818, 4819	1			4	5
*4804	1				5
HLA-B allele					
Well No.	1	2	3	4	5

<sup>1</sup>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.

<sup>2</sup>The nucleotide position, in the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> or 4<sup>th</sup> exon, 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, in the 2<sup>nd</sup>, 3<sup>rd</sup> or 4<sup>th</sup> 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](http://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*81 SSP primer set									
					Well				
					1	2	3	4	5
				Prod. No.:	200417801	200417802	200417803	200627704	200742305
	IHWC 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	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<sup>®</sup> HLA-B\*81 SSP**

**Product number:** 101.553-06 – including Taq polymerase  
101.553-06u – without Taq polymerase

**Lot number:** 18E

**Expiry date:** 2010-March-01

**Number of tests:** 6

**Number of wells per test:** 5

#### **Well specifications:**

Well No.	Production No.
1	2004-178-01
2	2004-178-02
3	2004-178-03
4	2006-277-04
5	2007-423-05

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

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

**Date of approval:** 2007-March-20

**Approved by:**

**Quality Control, Supervisor**

Lot No.: **18E**

Lot-specific information

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## Declaration of Conformity

**Product name:** Olerup SSP<sup>®</sup> HLA-B\*81  
**Product number:** 101.553-06, 101.553-06u  
**Lot number:** 18E

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

**Manufacturer:** Olerup SSP AB  
Hasselstigen 1  
SE-133 33 Saltsjöbaden, Sweden  
**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  
2008-March-20

Olle Olerup  
Managing Director

HLA-B\*81

Product Insert

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101.553-06u – without *Taq* polymerase

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Product Insert

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