

Lot No.: **45F**

Lot-specific information

## **Olerup SSP<sup>®</sup> HLA-B\*67**

Product number: 101.550-06u – without *Taq* polymerase  
Lot number: 45F  
Expiry date: 2011-January-01  
Number of tests: 6  
Number of wells per test: 4  
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. 45F.**

### **CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP<sup>®</sup>* HLA-B\*67 LOT.**

The HLA-B\*67 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP<sup>®</sup>* HLA-B\*67 lot was made (**Lot No. Y34**).

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

Well	5'-primer	3'-primer	rationale
4	-	Modified	Increased specificity of specific primer pair.

Lot No.: **45F**

Lot-specific information

## PRODUCT DESCRIPTION

### HLA-B\*67 SSP typing

#### CONTENT

The primer set contains 5'- and 3'-primers for identifying the B\*6701 to B\*6702 alleles.

#### PLATE LAYOUT

Each HLA-B\*67 test consists of 4 PCR reactions in an 8 well cut PCR plate. Wells 5 to 8 are empty.

1	2	3	4	empty	empty	empty	empty
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The 8 well PCR plate is marked with 'B67' in silver/gray ink.

Well No. 1 is marked with the Lot No. '45F'.

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\*67 SSP subtypings will be influenced by most B\*07, two B\*15, the B\*3576, the B\*38, most B39, most B\*42, the B\*46, the B\*5510, two B\*56, the B\*5820, the B\*7301, the B\*81 and the B\*8301 alleles when present on the other haplotype.

#### UNIQUELY IDENTIFIED ALLELES

All the HLA-B\*67, i.e. **B\*6701 to B\*6702**, recognized by the HLA Nomenclature Committee in October 2008<sup>1</sup> will be amplified by the primers in the HLA-B\*67 SSP kit.

The HLA-B\*67 subtyping kit cannot distinguish the B\*670101 and B\*670102 alleles.

<sup>1</sup>HLA-B alleles listed on the IMGT/HLA web page 2008-October-10, release 2.23.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

#### RESOLUTION IN HOMO- AND HETEROZYGOTES

The two HLA-B\*67 alleles can be combined in 3 homozygous and heterozygous combinations. All these genotypes give rise to unique amplification patterns.

Lot No.: **45F**

Lot-specific information

## SPECIFICITY TABLE

### HLA-B\*67 SSP subtyping

Specificities and sizes of the PCR products of the 4 primer mixes used for HLA-B\*67 SSP subtyping

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-B*67 alleles	Other amplified HLA-B alleles <sup>3</sup>
<b>1<sup>4</sup></b>	210 bp	<b>800 bp</b>	670101-670102	070201-070207, 0704-0707, 0709, 0711, 0712, 0714, 0715, 0717-0726, 0728, 0730, 0731, 0733-0736, 0739-0749N, 0751-0764, 0766-0768, 4201, 4204-4206, 4208, 5510, 8101-8104N
<b>2</b>	325 bp	1070 bp	6702	
<b>3</b>	165 bp	<b>800 bp</b>	670101-6702	380101-3807, 3809, 3811-3817, 39010101-39010102L, 390103-390602, 3908-391302, 3915, 3916, 3918-3920, 3922-3924, 3926-3928, 3931, 3935, 3937-3942, 3944, 3945, 5820
<b>4</b>	160 bp	1070 bp		070201-070202, 070204-070207, 0704-0707, 0709-0715, 0717-0726, 0728-0731, 0733-0736, 0739-0746, 0748, 0749N, 0751-0755, 0757-0768, 1576, 9501, 3576, 4201, 4202, 4204-4206, 4208, 4209, 460101, 460102-4602 <sup>weakly</sup> , 4603-4618, 5603, 5606, 7301 <sup>weakly</sup> , 8101-8104N, 8301

Lot No.: **45F**

**Lot-specific information**

<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\*67 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 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.

Wells number 1 and 3 contain 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 some non-HLA-B\*67 alleles will be amplified by primer mixes 1, 3 and 4.

<sup>4</sup>Primer mix 1 has a tendency of giving rise to nonspecific amplifications.

Lot No.: 45F

Lot-specific information

<b>INTERPRETATION TABLE</b>					
<b>HLA-B*67 SSP subtyping</b>					
<b>Amplification patterns of the B*67 alleles</b>					
	<b>Well</b>				
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	
Length of spec.	210	325	165	160	Length of spec.
PCR product					PCR product
Length of int.	800	1070	800	1070	Length of int.
pos. control <sup>1</sup>					pos. control <sup>1</sup>
5'-primer <sup>2</sup>	103	44	419	165	5'-primer <sup>2</sup>
	5'-CCT <sup>3'</sup>	5'-ggC <sup>3'</sup>	5'-gTT <sup>3'</sup>	5'-ACC <sup>3'</sup>	
3'-primer <sup>3</sup>	272	201	544	282	3'-primer <sup>3</sup>
	5'-TgT <sup>3'</sup>	5'-CTT <sup>3'</sup>	5'-ggT <sup>3'</sup>	5'-gCC <sup>3'</sup>	
Well No.	1	2	3	4	Well No.
HLA-B allele					HLA-B allele
*670101-670102	1		3		*670101-670102
*6702		2	3		*6702
*070201-070202, 070204-070207, 0704-0707, 0709, 0711, 0712, 0714, 0715, 0717-0726, 0728, 0730, 0731, 0733-0736, 0739-0746, 0748, 0749N, 0751-0755, 0757-0764, 0766-0768, 4201, 4204-4206, 4208, 8101-8104N	1			4	*070201-070202, 070204-070207, 0704-0707, 0709, 0711, 0712, 0714, 0715, 0717-0726, 0728, 0730, 0731, 0733-0736, 0739-0746, 0748, 0749N, 0751-0755, 0757-0764, 0766-0768, 4201, 4204-4206, 4208, 8101-8104N
*070203, 0747, 0756, 5510	1				*070203, 0747, 0756, 5510
*0710, 0713, 0729, 0765, 1576, 9501, 3576, 4202, 4209, 460101, 4603-4618, 5603, 5606, 8301				4	*0710, 0713, 0729, 0765, 1576, 9501, 3576, 4202, 4209, 460101, 4603-4618, 5603, 5606, 8301
*380101-3807, 3809, 3811-3817, 39010101-39010102L, 390103-390602, 3908-391302, 3915, 3916, 3918-3920, 3922-3924, 3926-3928, 3931, 3935, 3937-3942, 3944, 3945, 5820			3		*380101-3807, 3809, 3811-3817, 39010101-39010102L, 390103-390602, 3908-391302, 3915, 3916, 3918-3920, 3922-3924, 3926-3928, 3931, 3935, 3937-3942, 3944, 3945, 5820
*460102-4602, 7301				w	*460102-4602, 7301
HLA-B allele					HLA-B allele
Well No.	1	2	3	4	Well No.

Lot No.: **45F**

**Lot-specific information**

<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.

Wells number 1 and 3 contain 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> or 3<sup>rd</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> or 3<sup>rd</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.

Lot No.: **45F**

Lot-specific information

<b>CELL LINE VALIDATION SHEET</b>									
<b>HLA-B*67 SSP primer set</b>									
					<b>Well</b>				
					<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	
					Prod. No.:	200854801	200854802	200854803	200854804
<b>IHWC cell line</b>			<b>B*</b>						
1	9001	SA	*0702		+	-	-	+	
2	9280	LK707	*5201	*7301	-	-	-	w	
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	PTOUT	*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	-	-	-	-	

Lot No.: **45F**

Lot-specific information

## CERTIFICATE OF ANALYSIS

### **Olerup SSP<sup>®</sup> HLA-B\*67 SSP**

**Product number:** 101.550-06u – without *Taq* polymerase  
**Lot number:** 45F  
**Expiry date:** 2011-January-01  
**Number of tests:** 6  
**Number of wells per test:** 4

#### **Well specifications:**

Well No.	Production No.
1	2008-548-01
2	2008-548-02
3	2008-548-03
4	2008-548-04

The specificity of each primer solution of the HLA-B\*67 primer set has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solution 2 were available. The specificities of the primers in primer solution 2 was tested by separately adding one additional 5'-primer, respectively one additional 3'-primer.

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

**Date of approval:** 2009-May-27

**Approved by:**

**Quality Control, Supervisor**



Lot No.: **45F**

Lot-specific information

## Declaration of Conformity

**Product name:** *Olerup* SSP® HLA-B\*67  
**Product number:** 101.550-06u  
**Lot number:** 45F

**Intended use:** HLA-B\*67 high resolution histocompatibility testing

**Manufacturer:** *Olerup* SSP AB  
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**Phone:** +46-8-717 88 27  
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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.

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

Lot No.: **45F**

Lot-specific information

Lot No.: **45F**

Lot-specific information

Lot No.: **45F**

Lot-specific information

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