

Olerup SSP[®] HLA-B*67

Product number:	101.550-06u – without <i>Taq</i> polymerase
Lot number:	49M
Expiry date:	2014-January-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. 49M.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP[®]* 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[®]* HLA-B*67 lot was made (**Lot No. 16K**).

Two wells have been added to the HLA-B*67 kit,
wells **5 and 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
1	Modified	Modified	Improved specificity of primer pair.
5	New	New	Improved allelic resolution.
6	New	New	New primer pair for the B*67:03 allele.

PRODUCT DESCRIPTION

HLA-B*67 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*67:01 to B*67:03 alleles.

PLATE LAYOUT

Each HLA-B*67 test consists of 4 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 'B67' in silver/gray ink.

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

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*67 SSP subtypings will be influenced by most B*07, eight B*14, five B*15, most B*18, eight B*27, four B*35, the B*37:11, the B*38, most B*39, several B*40, most B*42, the B*46, the B*48, two B*54, two B*55, two B*56, the B*58:20, the B*73, the B*81 and the B*83:01 alleles when present on the other haplotype. In addition, the C*07:102 allele will be weakly amplified by primer mix 5 and the C*06:40 and C*07:137:01-07:137:02 alleles will be amplified by primer mix 6.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*67, i.e. **B*67:01 to B*67:03**, recognized by the HLA Nomenclature Committee in April 2011¹ will be amplified by the primers in the HLA-B*67 SSP kit. The HLA-B*67 subtyping kit cannot distinguish the B*67:01:01 and B*67:01:02 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2011-April-08, release 3.4.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 4 alleles generate 3 amplification patterns that can be combined in 6 homozygous and heterozygous combinations. 2 of these genotypes do not give rise to unique amplification patterns.

+ - + - + + *67:01:01, *67:03 = *67:03, *67:03

*67:01:01 = *67:01:01-67:01:02

SPECIFICITY TABLE

HLA-B*67 SSP subtyping

Specificities and sizes of the PCR products of the 6 primer mixes used for HLA-B*67 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*67 alleles	Other amplified HLA-B alleles ³
1	210 bp	800 bp	*67:01:01-67:01:02, 67:03	*07:02:01-07:02:20, 07:04-07:07, 07:09, 07:11-07:12, 07:14-07:15, 07:17-07:26, 07:28, 07:30-07:31, 07:33-07:36, 07:39-07:49N, 07:51-07:64, 07:66-07:68:02, 07:73-07:82, 07:84, 07:87-07:124, 07:126-07:130, 38:26, 42:01:01-42:02, 42:04-42:06, 42:08-42:10, 42:12-42:16, 54:02, 55:10, 81:01-81:04N
2	325 bp	1070 bp	*67:02	
3⁵	165 bp	800 bp	*67:01:01-67:03	*38:01:01, 38:01:03-38:07, 38:09, 38:11-38:21, 38:23-38:29, 39:01:01:01-39:01:01:02L, 39:01:03-39:06:02, 39:08-39:13:02, 39:15-39:16, 39:18-39:20, 39:22-39:24:02, 39:26-39:28, 39:31, 39:35, 39:37-39:42, 39:44-39:46, 39:48-39:49, 39:51-39:63, 58:20
4	160 bp	1070 bp		*07:02:01-07:02:02, 07:02:04-07:02:20, 07:04-07:05:03, 07:05:05-07:07, 07:09-07:15, 07:17-07:26, 07:28-07:31, 07:33-07:36, 07:39-07:46, 07:48-07:49N, 07:51-07:55, 07:57-07:68:02, 07:70-07:72, 07:74-07:84, 07:86-07:108, 07:110-07:130, 15:76, 15:101, 27:75, 35:76, 42:01:01-42:02, 42:04-42:06, 42:08-42:10, 42:12-42:16, 46:01:01-46:27, 54:01:02, 55:35, 56:03, 56:06, 73:01 ^w -73:02 ^w , 81:01-81:05, 83:01

5	240 bp	1070 bp	*67:01:01-67:03	*07:02:01-07:10, 07:12-07:26, 07:28, 07:30-07:35, 07:37, 07:39-07:56, 07:58-07:64, 07:66-07:69, 07:73-07:74, 07:76-07:80, 07:82-07:85, 07:87-07:130, 14:01:01-14:01:02, 14:07N-14:08, 14:10, 14:12, 14:14, 14:19, 15:68, 15:71, 15:175, 18:01:01-18:01:12, 18:02-18:03, 18:05-18:08, 18:10-18:15, 18:17N-18:24, 18:26-18:48, 18:50-18:53, 18:55-18:63, 27:08, 27:12, 27:18, 27:26, 27:33, 27:40, 27:44, 35:50, 35:84, 35:162, 37:11, 39:01:01:01-39:01:01:02L, 39:01:03-39:03, 39:05:01-39:11, 39:13:01-39:19:02, 39:22-39:24:01, 39:25N-39:62, 40:02:01-40:06:03, 40:08-40:09, 40:11:01-40:11:02, 40:18, 40:20, 40:24, 40:26-40:29, 40:35, 40:39-40:40, 40:44, 40:50, 40:56, 40:64, 40:68, 40:70-40:71, 40:74-40:75, 40:78, 40:82-40:83, 40:85-40:86, 40:89-40:91, 40:93-40:95, 40:97-40:99, 40:103-40:105, 40:107, 40:111, 40:115, 40:119-40:120, 40:122, 40:127, 40:131, 40:133Q, 40:142N-40:145, 40:148-40:149, 40:159, 40:161-40:162, 42:01:01-42:02, 42:04-42:14, 42:16, 48:01:01-48:17, 48:19-48:21, 48:23-48:25, 54:02, 55:10, 73:01-73:02, 81:01-81:05, C*07:102^w
6⁴	110 bp	1070 bp	*67:03	C*06:40, C*07:137:01-07:137:02

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*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.

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.

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.

³Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B*67 alleles will be amplified by primer mixes 1, 3 to 5. In addition, the C*07:102 allele will be weakly amplified by primer mix 5 and the C*06:40 and C*07:137:01-07:137:02 alleles will be amplified by primer mix 6.

⁴Specific PCR fragments shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR bands.

⁵Primer mix 3 may give a lower yield of specific PCR product than other HLA-B*67 primer mixes. “w”, may be weakly amplified.

INTERPRETATION TABLE						
HLA-B*67 SSP subtyping						
Amplification patterns of the B*67 alleles						
	Well					
	1	2	3	4	5	6
Length of spec.	210	325	165	160	240	110
PCR product						
Length of int.	800	1070	800	1070	1070	1070
pos. control¹						
5'-primer²	103	44	419	165	103	477
	5' -CCT 3'	5' -ggC 3'	5' -gTT 3'	5' -ACC 3'	5' -CCT 3'	5' -gCC 3'
	103				103	
	5' -CCT 3'				5' -CCT 3'	
3'-primer³	272	201	544	282	302	544
	5' -TgT 3'	5' -CTT 3'	5' -ggT 3'	5' -gCC 3'	5' -ggC 3'	5' -ggT 3'
Well No.	1	2	3	4	5	6
HLA-B allele						
*67:01:01-67:01:02	1		3		5	
*67:02		2	3		5	
*67:03	1		3		5	6
*07:02:01-07:02:02, 07:02:04-07:02:20, 07:04-07:05:03, 07:05:05-07:07, 07:09, 07:12, 07:14-07:15, 07:17-07:26, 07:28, 07:30-07:31, 07:33-07:35, 07:39-07:46, 07:48-07:49N, 07:51-07:55, 07:58-07:64, 07:66-07:68:02, 07:74, 07:76-07:80, 07:82, 07:84, 07:87-07:108, 07:110-07:124, 07:126-07:130, 42:01:01-42:02, 42:04-42:06, 42:08-42:10, 42:12-42:14, 42:16, 81:01-81:04N	1			4	5	
*07:02:03, 07:05:04, 07:47, 07:56, 07:73, 07:109, 54:02, 55:10	1				5	
Well No.	1	2	3	4	5	6

Lot No.: **49M**

Lot-specific information

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Length of spec.	210	325	165	160	240	110
PCR product						
Well No.	1	2	3	4	5	6
*07:03, 07:08, 07:16, 07:32, 07:37, 07:50, 07:69, 07:85, 14:01:01-14:01:02, 14:07N-14:08, 14:10, 14:12, 14:14, 14:19, 15:68, 15:71, 15:175, 18:01:01-18:01:12, 18:02-18:03, 18:05-18:08, 18:10-18:15, 18:17N-18:24, 18:26-18:48, 18:50-18:53, 18:55-18:63, 27:08, 27:12, 27:18, 27:26, 27:33, 27:40, 27:44, 35:50, 35:84, 35:162, 37:11, 39:07, 39:14, 39:17, 39:25N, 39:29-39:30, 39:32-39:34, 39:36, 39:43, 39:47, 39:50, 40:02:01-40:06:03, 40:08-40:09, 40:11:01-40:11:02, 40:18, 40:20, 40:24, 40:26-40:29, 40:35, 40:39-40:40, 40:44, 40:50, 40:56, 40:64, 40:68, 40:70-40:71, 40:74-40:75, 40:78, 40:82-40:83, 40:85-40:86, 40:89-40:91, 40:93-40:95, 40:97-40:99, 40:103-40:105, 40:107, 40:111, 40:115, 40:119-40:120, 40:122, 40:127, 40:131, 40:133Q, 40:142N-40:145, 40:148-40:149, 40:159, 40:161-40:162, 42:07, 42:11, 48:01:01-48:17, 48:19-48:21, 48:23-48:25					5	
*07:10, 07:13, 07:83, 07:125, 81:05				4	5	
*07:11, 07:36, 07:57, 07:75, 07:81, 42:15	1			4		
*07:29, 07:65, 07:70-07:72, 07:86, 15:76, 15:101, 27:75, 35:76, 46:01:01-46:27, 54:01:02, 55:35, 56:03, 56:06, 83:01				4		
*38:01:01, 38:01:03-38:07, 38:09, 38:11-38:21, 38:23-38:25, 38:27-38:29, 39:04, 39:12, 39:20, 39:24:02, 39:63, 58:20			3			
*38:26	1		3			
*39:01:01:01-39:01:01:02L, 39:01:03-39:03, 39:05:01-39:06:02, 39:08-39:11, 39:13:01-39:13:02, 39:15-39:16, 39:18-39:19:02, 39:22-39:24:01, 39:26-39:28, 39:31, 39:35, 39:37-39:42, 39:44-39:46, 39:48-39:49, 39:51-39:62			3		5	
*73:01-73:02				w	5	
C*06:40, C*07:137:01-07:137:02						6
C*07:102					w	
HLA-B allele						
Well No.	1	2	3	4	5	6

Lot No.: **49M**

Lot-specific information

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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.

Wells number 1 and 3 contain 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.

“w”, may be weakly amplified.

CELL LINE VALIDATION SHEET					
HLA-B*67 SSP primer set					
					Well
				Prod. No.:	
					1 2 3 4 5 6
					201189201 200854802 201189203 200854804 201189205 201189206
IHC cell line			B*		
1	9001 SA	*07:02			+ - - + + -
2	9280 LK707	*52:01	*73:01		- - - W + -
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 S8TO	*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*67 SSP

Product number: 101.550-06u – without *Taq* polymerase
Lot number: 49M
Expiry date: 2014-January-01
Number of tests: 6
Number of wells per test: 4

Well specifications:

Well No.	Production No.
1	2011-892-01
2	2008-548-02
3	2011-892-03
4	2008-548-04
5	2011-892-05
6	2011-892-06

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 solutions 2 and 6 were available. The specificities of the primers in primer solutions 2 and 6 were 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: 2011-September-08

Approved by:

Quality Control, Supervisor

Lot No.: **49M**

Lot-specific information

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Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*67

Product number: 101.550-06u

Lot number: 49M

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

Manufacturer: *Olerup* SSP AB
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SE-112 51 Stockholm, Sweden
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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, Franzengatan 5, SE-112 51 Stockholm, 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.)

Stockholm, Sweden
2011-September-08

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

Lot No.: **49M**

Lot-specific information

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