

Olerup SSP[®] HLA-B*47

Product number:	101.545-06u – without <i>Taq</i> polymerase
Lot number:	68M
Expiry date:	2014-March-01
Number of tests:	6
Number of wells per test:	8
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. 68M.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP[®]* HLA-B*47 LOT.

The HLA-B*47 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP[®]* HLA-B*47 lot was made (**Lot No. 10K**).

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	Added	Added	Primer pair added for the B*47:08 allele, exchanged positive control primer pair.
8	Added	Added	Primer pair added for the B*47:08 allele.

PRODUCT DESCRIPTION

HLA-B*47 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*47:01 to B*47:08 alleles.

PLATE LAYOUT

Each HLA-B*47 test consists of 8 PCR reactions in an 8 well cut PCR plate.

1	2	3	4	5	6	7	8
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The 8 well PCR plate is marked with ‘B47’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘68M’.

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*47 SSP subtypings will be influenced by the B*07:133, the B*08:14, B*13:32, fourteen B*15, three B*18, most B*27, five B*35, two B*37, most B*40, the B*41, most B*44, the B*45, the B*48:21, most B*49, the B*50, the B*51:112, two B*57 and the B*82 alleles when present on the other haplotype. In addition, the A*02:298 allele will be amplified by primer mixes 4 and 8, C*16:19 allele will be amplified by primer mix 5 and the A*23.23 allele will be amplified by primer mix 6.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*47, i.e. **B*47:01 to B*47:08**, recognized by the HLA Nomenclature Committee in July 2011¹ will be amplified by the primers in the HLA-B*47 SSP kit. The HLA-B*47 kit cannot distinguish the B*47:01:01:01-47:01:02 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2011-July-14, release 3.5.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 10 alleles generate 8 amplification patterns that can be combined in 36 homozygous and heterozygous combinations. 20 of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products were not considered in these calculations.

+++--+-- *47:01:01:01, *47:02 = *47:02, *47:06
+-+---+ *47:03, *47:08 = *47:06, *47:08
++-+-++ *47:04, *47:07 = *47:04, *47:08 = *47:05, *47:08
+-+--+ *47:01:01:01, *47:08 = *47:07, *47:08 = *47:08, *47:08
+-+---+ *47:03, *47:05 = *47:05, *47:06
+-+--+ *47:03, *47:07 = *47:06, *47:07
+-+--+ *47:01:01:01, *47:03 = *47:01:01:01, *47:06 = *47:03, *47:06 = *47:06,
*47:06
+-+---+ *47:01:01:01, *47:07 = *47:07, *47:07

*47:01:01:01 = *47:01:01:01-47:01:02

SPECIFICITY TABLE

HLA-B*47 SSP subtyping

Specificities and sizes of the PCR products of the 8 primer mixes used for HLA-B*47 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*47 alleles	Other amplified HLA-B alleles ³
1	135 bp	800 bp	*47:01:01:01-47:01:02, 47:05-47:08	*37:06
2	150 bp	1070 bp	*47:01:01:01-47:01:02, 47:03-47:08	*37:06, 40:13, 40:19, 40:47, 40:96, 40:109-40:110, 40:117, 40:157, 44:02:01:01-44:02:12, 44:02:14-44:05:03, 44:07, 44:10-44:39, 44:41:01-44:43:02, 44:45, 44:47-44:56N, 44:58N-44:59, 44:61N-44:74, 44:76-44:82, 44:84-44:89, 44:91-44:126, 44:128, 44:132-44:133, 49:01:01-49:02, 49:04-49:20, 51:112, 57:45, 57:51
3	150 bp	1070 bp	*47:02	*07:133, 15:46, 15:53, 15:106, 15:143, 15:212, 18:48, 35:19, 35:47, 35:63, 35:154, 40:01:01-40:11:02, 40:14:01-40:16, 40:18, 40:20, 40:22N-40:40, 40:42-40:45, 40:48-40:78, 40:80-40:92, 40:94-40:95, 40:97-40:108, 40:111-40:116, 40:118N-40:136, 40:138-40:156, 40:158-40:173, 41:01-41:13, 41:15-41:19, 44:09, 44:46, 44:75, 44:90, 44:129, 44:131, 45:01-45:13, 50:01:01-50:02, 50:04-50:05, 50:07-50:14
4⁵	145 bp, 180 bp	800 bp	*47:04, 47:08	*08:14, 27:15, 27:28, 27:62, 27:71, 40:47, 40:96, 40:110, 40:157, 44:02:01:01-44:02:12, 44:02:14-44:02:18, 44:02:20-44:05:02, 44:07, 44:10, 44:12-44:17, 44:19N-44:24, 44:26-44:39, 44:41:01-44:43:02, 44:45, 44:47-44:49, 44:51-44:56N, 44:58N-44:59, 44:61N-44:74, 44:76-44:82, 44:84-44:89, 44:91-44:94, 44:96-44:128, 44:132-44:133, 49:02, A*02:298

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5	135 bp	800 bp	*47:02-47:03, 47:06	*07:133, 13:32, 15:46, 15:53, 15:106, 15:143, 15:145, 15:176, 15:212, 18:31, 18:48, 35:19, 35:47, 35:63, 35:154, 40:01:01-40:01:04, 40:01:06- 40:11:02, 40:14:01-40:16, 40:18, 40:20, 40:22N-40:36, 40:38-40:40, 40:42-40:45, 40:48-40:92, 40:94- 40:95, 40:97-40:108, 40:111-40:116, 40:118N-40:134, 40:136, 40:138- 40:156, 40:158-40:173, 41:01-41:19, 44:09, 44:46, 44:90, 44:129, 44:131, 45:01-45:13, 50:01:01-50:02, 50:04- 50:05, 50:07-50:14, C*16:19
6	135 bp	1070 bp	*47:01:01:01- 47:03, 47:06- 47:08	*15:06, 15:27:01-15:27:03, 15:84, 15:109, 15:195, 18:27, 82:01-82:02, A*23:23
7	235 bp	1070 bp	*47:04-47:05	*27:01-27:05:15, 27:05:17-27:06, 27:08-27:10, 27:12-27:13, 27:16- 27:18, 27:20, 27:23, 27:26-27:27, 27:29, 27:31, 27:35-27:37, 27:39- 27:42, 27:44-27:46, 27:48-27:61, 27:64N-27:69, 27:72-27:75, 27:77- 27:80, 27:82, 37:02, 48:21
8^{4,6}	105 bp, 180 bp	800 bp	*47:07-47:08	*08:14, 27:15, 27:28, 27:62, 27:71, 35:138, 44:26, A*02:298

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*47 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, 4, 5 and 8 contain 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.

³Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B*47 alleles will be amplified by all primer mixes. In addition, the A*02:298 allele will be amplified by primer mixes 4 and 8, C*16:19 allele will be amplified by primer mix 5 and the A*23:23 allele will be amplified by primer mix 6.

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⁴Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁵Primer mix 4: Specific PCR product of 145 bp in the B*47:04 and in the B*40:47, 40:96, 40:110, 40:157, 44:02:01:01-44:02:12, 44:02:14-44:02:18, 44:02:20- 44:03:14, 44:05:01-44:05:02, 44:07, 44:10, 44:12-44:17, 44:19N-44:24, 44:26-44:39, 44:41:01-44:43:02, 44:45, 44:47-44:49, 44:51-44:55, 44:58N-44:59, 44:61N-44:74, 44:76-44:82, 44:84-44:89, 44:91-44:94, 44:96-44:128, 44:133 and 49:02 alleles. Specific PCR product of 180 bp in the B*47:08 and in the B*08:14, 27:15, 27:28, 27:62 and 27:71 and the A*02:298 alleles.

Specific PCR product of 145 and 180 bp in the B*44:04, 44:56N and 44:132 alleles.

⁶Primer mix 8: Specific PCR product of 105 bp in the B*47:07 and the B*35:138 and 44:26 alleles. Specific PCR product of 180 bp in the B*47:08 and in the B*08:14, 27:15, 27:28, 27:62 and 27:71 and the A*02:298 alleles.

INTERPRETATION TABLE								
HLA-B*47 SSP subtyping								
Amplification patterns of the B*47:01 to 47:08 alleles								
	Well⁴							
	1	2	3	4	5	6	7	8
Length of spec.	135	150	150	145	135	135	235	105
PCR product(s)				180				180
Length of int.	800	1070	1070	800	800	1070	1070	800
pos. control ¹								
5'-primer ²	206	206	206	206	206	368	363	106
	5'-gAA 3'	5'-gAA 3'	5'-gAA 3'	5'-gAA 3'	5'-gAA 3'	5'-gTT 3'	5'-AAT 3'	5'-CCA 3'
				418	523			418
				5'-Agg 3'	5'-CCg 3'			5'-Agg 3'
3'-primer ³	301	317	317	309	301	463	559	171
	5'-gTC 3'	5'-ggA 3'	5'-CgC 3'	5'-gTg 3'	5'-gCT 3'	5'-gCT 3'	5'-CTC 3'	5'-ACC 3'
				559	610			559
				5'-CgT 3'	5'-CTg 3'			5'-CgT 3'
Well No.	1	2	3	4	5	6	7	8
HLA-B allele								
*47:01:01:01-47:01:02	1	2				6		
*47:02			3		5	6		
*47:03		2			5	6		
*47:04		2		4			7	
*47:05	1	2					7	
*47:06	1	2			5	6		
*47:07	1	2				6		8
*47:08	1	2		4		6		8
*07:133, 15:46, 15:53, 15:106, 15:143, 15:212, 18:48, 35:19, 35:47, 35:63, 35:154, 40:01:01-40:01:04, 40:01:06-40:11:02, 40:14:01-40:16, 40:18, 40:20, 40:22N-40:36, 40:38-40:40, 40:42-40:45, 40:48-40:78, 40:80-40:92, 40:94-40:95, 40:97-40:108, 40:111-40:116, 40:118N-40:134, 40:136, 40:138-40:156, 40:158-40:173, 41:01-41:13, 41:15-41:19, 44:09, 44:46, 44:90, 44:129, 44:131, 45:01-45:13, 50:01:01-50:02, 50:04-50:05, 50:07-50:14			3		5			
Well No.	1	2	3	4	5	6	7	8

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Length of spec.	135	150	150	145	135	135	235	105
PCR product				180				180
Well No.	1	2	3	4	5	6	7	8
*08:14, 27:15, 27:28, 27:62, 27:71, A*02:298				4				8
*13:32, 15:145, 15:176, 18:31, 40:79, 41:14, C*16:19					5			
*15:06, 15:27:01-15:27:03, 15:84, 15:109, 15:195, 18:27, 82:01- 82:02, A*23:23						6		
*27:01-27:05:15, 27:05:17-27:06, 27:08-27:10, 27:12-27:13, 27:16- 27:18, 27:20, 27:23, 27:26-27:27, 27:29, 27:31, 27:35-27:37, 27:39- 27:42, 27:44-27:46, 27:48-27:61, 27:64N-27:69, 27:72-27:75, 27:77- 27:80, 27:82, 37:02, 48:21							7	
*35:138								8
*37:06	1	2						
*40:01:05, 40:37, 40:135, 44:75			3					
*40:13, 40:19, 40:109, 40:117, 44:02:19, 44:05:03, 44:11, 44:18, 44:25, 44:50, 44:95, 49:01:01- 49:01:03, 49:04-49:20, 51:112, 57:45, 57:51		2						
*40:47, 40:96, 40:110, 40:157, 44:02:01:01-44:02:12, 44:02:14- 44:02:18, 44:02:20-44:05:02, 44:07, 44:10, 44:12-44:17, 44:19N-44:24, 44:27:01-44:39, 44:41:01-44:43:02, 44:45, 44:47- 44:49, 44:51-44:56N, 44:58N- 44:59, 44:61N-44:74, 44:76- 44:82, 44:84-44:89, 44:91-44:94, 44:96-44:126, 44:128, 44:132- 44:133, 49:02		2		4				
*44:26		2		4				8
*44:127				4				
HLA-B allele								
Well No.	1	2	3	4	5	6	7	8

Lot No.: **68M**

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, 4, 5 and 8 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

²The 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.

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.

⁴Primer mix 4: Specific PCR product of 145 bp in the B*47:04 and in the B*40:47, 40:96, 40:110, 40:157, 44:02:01:01-44:02:12, 44:02:14-44:02:18, 44:02:20- 44:03:14, 44:05:01-44:05:02, 44:07, 44:10, 44:12-44:17, 44:19N-44:24, 44:26-44:39, 44:41:01-44:43:02, 44:45, 44:47-44:49, 44:51-44:55, 44:58N-44:59, 44:61N-44:74, 44:76-44:82, 44:84-44:89, 44:91-44:94, 44:96-44:128, 44:133 and 49:02 alleles. Specific PCR product of 180 bp in the B*47:08 and in the B*08:14, 27:15, 27:28, 27:62 and 27:71 and the A*02:298 alleles.

Specific PCR product of 145 and 180 bp in the B*44:04, 44:56N and 44:132 alleles.

Primer mix 8: Specific PCR product of 105 bp in the B*47:07 and the B*35:138 and 44:26 alleles. Specific PCR product of 180 bp in the B*47:08 and in the B*08:14, 27:15, 27:28, 27:62 and 27:71 and the A*02:298 alleles.

CELL LINE VALIDATION SHEET												
HLA-B*47 SSP primer set												
				Well								
				1	2	3	4	5	6	7	8	
				Prod. No.:	200959201	200959202	200959203	201190504	201072505	200959206	200959207	201190508
IHC 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*47 SSP

Product number: 101.545-06u – without *Taq* polymerase
Lot number: 68M
Expiry date: 2014-March-01
Number of tests: 6
Number of wells per test: 8

Well specifications:

Well No.	Production No.
1	2009-592-01
2	2009-592-02
3	2009-592-03
4	2011-905-04
5	2010-725-05
6	2009-592-06
7	2009-592-07
8	2011-905-08

The specificity of each primer solution has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solution 8 were available. The specificity of the primers in primer solution 8 were tested by separately adding additional 5'-primers, respectively one additional 3'-primer. In primer solution 5 one of the 5'-primers was not possible to test, and in primer solution 8 one of the 3'-primers was not possible to test.

In addition, one 5'-primer and one 3'-primer in primer solution 4 and one 3'-primer in primer solution 5 were tested by separately adding one additional 3'-primer respective one additional 5'-primer.

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

Date of approval: 2011-October-06

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*47
Product number: 101.545-06u
Lot number: 68M

Intended use: HLA-B*47 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Franzengatan 5
SE-112 51 Stockholm, 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 9001:2008 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.

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-October-06

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

Lot No.: **68M**

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