

101.572-12– including *Taq* pol., IFU-01
101. 572-12u– without *Taq* pol., IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **10V**

Lot-specific information
Olerup SSP® HLA-B*57:01

Product number:	101.572-12 – including <i>Taq</i> polymerase 101.572-12u – without <i>Taq</i> polymerase
Lot number:	10V
Expiry date:	2016-June-01
Number of tests:	12
Number of wells per test:	15+1
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. 10V.

Complete product documentation consists of generic Instructions for Use (IFU), lot specific Product Insert, Worksheet and Certificate.

**CHANGES COMPARED TO THE PREVIOUS OLERUP SSP®
HLA-B*57:01 LOT (02S).**

The format of the Product Insert and Worksheet have been changed.

The HLA-B*57:01 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP®* HLA-B*57:01 lot was made (**Lot No. 02S**).

As of lot series V, the Specificity Table is included in the lot-specific Product Insert, and the Interpretation Table is included in the Worksheet.

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	3'-primer added for the B*57:62 allele.
5	-	Added	3'-primer added for the B*57:64 allele.
6	-	Added	3'-primer added for the B*57:65 allele.

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Well **16** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup SSP*® HLA Class I, DRB, DQB1 and DPB1 amplicons as well as amplicons generated by control primer pairs.

PCR product sizes range from 75 to 430 base pairs.
The PCR product generated by the control primer pair is 430 base pairs.

Length of PCR product	105	200	105	80	75	80
5'-primer¹	164	340	440	45	45	43
	5'-CAC ³	5'-Agg ³	5'-TTA ³	5'-Tgg ³	5'-Tgg ³	5'-Tgg ³
3'-primer²	231	2nd I	507	59	58	57
	5'-TgC ³	5'-AAA ³	5'-TTg ³	5'-CTC ³	5'-ggC ³	5'-CTC ³
A*	+	+	+			
B*	+	+	+			
C*	+	+	+			
DRB1				+	+	
DRB3				+	+	
DRB5				+		
DQB1					+	
DPB1						+

¹The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

²The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and codon 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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PRODUCT DESCRIPTION

HLA-B*57:01 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*57:01:01 to 57:01:15 alleles.

PLATE LAYOUT

Each test consists of 16 PCR reactions in a 16 well cut PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	NC

Wells 1 to 15 – HLA-B*57:01 primers.

Well 16 – Negative Control (NC).

The 16 well cut PCR plate is marked with ‘B*57:01’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘10V’.

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 16 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

Due to the sharing of sequence motifs between HLA-B alleles, non-HLA-B*57:01 alleles will be amplified by primer mixes 1 to 7 and 9 to 14. In addition, a few HLA-A and HLA-C alleles will be amplified by primer mixes 5, 7, 10, 11 and 13 to 15.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

HLA-B*57:01 will give rise to a unique amplification pattern by the primers in the HLA-B*57:01 kit¹.

The HLA-B*57:01 typing kit cannot distinguish the silent mutations in the B*57:01:01 to B*57:01:15 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2013-July-25, release 3.13.1, www.ebi.ac.uk/imgt/hla.

²Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page <http://hla.alleles.org/alleles/deleted.html>.

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Lot-specific information
SPECIFICITY TABLE

HLA-B*57:01 SSP subtyping

Specificities and sizes of the PCR products of the 15+1 primer mixes used for HLA-B*57:01 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*57:01 alleles	Other amplified HLA-B alleles ^{3,4}
1⁵	90 bp	800 bp	*57:01:01-57:01:15	*57:02:01-57:15, 57:17-57:19, 57:21-57:35, 57:37-57:44, 57:46-57:50, 57:52-57:61, 57:63-57:65, 58:36
2	220 bp	800 bp	*57:01:01-57:01:15	*57:03:01-57:03:02, 57:06-57:08, 57:10, 57:14-57:18, 57:20-57:23, 57:25-57:27, 57:29, 57:31-57:41, 57:43-57:51, 57:53-57:60, 57:62, 57:64-57:65, 35:208, 40:30, 40:34, 44:153, 55:14, 58:14
3⁵	95 bp 170 bp 215 bp	800 bp		*57:04, 57:32, 44:153 *57:06, 57:18 *57:27
4⁵	100 bp	1070 bp		*57:15, 57:20, 57:29, 57:62, 07:120, 15:214, 18:81, 40:150
5⁵	90 bp 165 bp 200 bp 245 bp	800 bp		*57:16, 57:34 *57:23 *57:46, 57:64 *57:07, 57:26, 44:153, 55:14, C*06:72
6⁵	90 bp	1070 bp		*57:02:01-57:03:02, 57:07, 57:09, 57:12, 57:17, 57:39, 57:42, 57:46, 57:57, 57:63, 40:30, 40:34 *57:08, 57:65
7^{5,6}	205 bp 100 bp 140 bp 175 bp 215 bp 240 bp	1070 bp		*57:53 *57:13, 57:31, 40:30, 40:34, C*06:72 57:14, 57:50 35:208, 44:153, 55:14 58:14 *57:09, 57:24 *57:13, 57:25, 40:30, 40:34
8	185 bp	1070 bp		*57:10, 57:44
9⁵	110 bp 150 bp	800 bp		*57:33 *57:21, 57:40, 14:20, 35:127
10^{5,6}	90 bp 170 bp 205 bp 240 bp	1070 bp		*57:04, 57:41, 44:153, C*06:72 *57:37 57:13, 57:22, 57:57, 57:63, 55:14, C*06:72^w *57:43
11⁵	100 bp	1070 bp	*57:01:01-57:01:15	*57:06, 57:08, 57:10, 57:13-57:16, 57:18-57:27, 57:29-57:31, 57:33-57:38, 57:40 ^w , 57:41, 57:43-57:45,

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			57:47-57:52, 57:54-57:56, 57:58-57:62, 57:64-57:65, 35:208, 55:14, 58:14, C*06:72
12^{5,6}	75 bp 100 bp 140 bp	1070 bp	*57:35 *57:36 *57:08, 57:38, 57:60, 15:87
13⁵	105 bp 155 bp 200 bp	1070 bp	*57:45, 57:51, A*02:285 *57:58, 58:12, A*02:42, A*02:310 *57:49
14⁵	100 bp 125 bp	1070 bp	*57:59, 15:116, 40:63, 40:92, 44:169, C*03:129, C*17:07 *57:47, 57:54, 15:33, 15:248, 49:22, 51:126 C*03:87, C*05:27, C*05:39
15	145 bp 185 bp 295 bp	1070 bp	*57:56 *57:48, A*33:12 *57:55
16⁷	-	-	Negative control

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*57:01 SSP typings. When the primers in a primer mix can give rise to HLA-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits, the alleles listed are specified according to amplicon length.

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 internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

³For several HLA Class I alleles 1st and/or 4th exon(s) and beyond, as well as intron nucleotide sequences, are not available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

⁴Due to the sharing of sequence motifs between HLA-B alleles, non-HLA-B*57:01 alleles will be amplified by primer mixes 1 to 7 and 9 to 14. In addition, a few HLA-A and HLA-C alleles will be amplified by primer mixes 5, 7, 10, 11 and 13 to 15.

⁵HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁶Primer mixes 7 and 10 may have tendencies of unspecific amplifications.

⁷Well 16 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by control primer pairs. PCR product sizes range from 75 to 200 base pairs. The PCR product generated by the control primer pair is 430 base pairs.

'w', might be weakly amplified.

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	90	220	95	100	90	90	100	185	110	90	100	75
PCR product			170		165	205	140		150	170		100
			215		200		175			205		140
					245		215			240		
							240					
Length of int.	800	800	800	1070	800	1070	1070	1070	800	1070	1070	1070
pos. control ¹												
5'-primer(s) ²	209	362	362	209	130	320	362	103	352	362	362	209
	5'-ggC 3'	5'-ggT 3'	5'-ggT 3'	5'-ggC 3'	5'-AgT 3'	5'-CCC 3'	5'-ggT 3'	5'-CCT 3'	5'-ACg 3'	5'-ggT 3'	5'-ggT 3'	5'-ggC 3'
				704	200	362		122	353	878		
				5'-TgT 3'	5'-TCg 3'	5'-ggT 3'		5'-CCT 3'	5'-CAA 3'	5'-gCA 3'		
					209				392			
					5'-ggA 3'				5'-CgA 3'			
					362							
					5'-ggT 3'							
3'-primer(s) ³	256	539	418	259	256	2 nd I	421	256	463	409	419	244
	5'-CCC 3'	5'-TCA 3'	5'-gTC 3'	5'-CTT 3'	5'-CCC 3'	5'-TCg 3'	5'-ggT 3'	5'-CCC 3'	5'-gCT 3'	5'-ATA 3'	5'-Cgg 3'	5'-CTT 3'
			481	263	523	412	463			527	419	268
			5'-gTA 3'	5'-gTT 3'	5'-ACA 3'	5'-gTT 3'	5'-gCg 3'			5'-CCT 3'	5'-CAg 3'	5'-gTg 3'
			500	271	559	521	486			559		302
			5'-ggA 3'	5'-CAC 3'	5'-CgT 3'	5'-ggA 3'	5'-gCg 3'			5'-Cgg 3'		5'-ggg 3'
			537	774	572		505			916		302
			5'-Agg 3'	5'-ggT 3'	5'-gCg 3'		5'-gCC 3'			5'-gAC 3'		5'-ggC 3'
							538					320
							5'-gTC 3'					5'-Agg 3'
							559					
							5'-CTC 3'					

Well No.	13	14	15
Length of spec.	105	100	145
PCR product	155	125	185
	200		295
Length of int.	1070	1070	1070
pos. control ¹			
5'-primer(s) ²	97	467	106
	5'-TCg 3'	5'-CTg 3'	5'-CCA 3'
	142	485	395
	5'-TCT 3'	5'-CAA 3'	5'-gCC 3'
	193	499	757
	5'-CCA 3'	5'-TCT 3'	5'-CCA 3'
3'-primer(s) ³	256	559	212
	5'-CCC 3'	5'-CAg 3'	5'-ggT 3'
			539
			5'-TCA 3'
			916
			5'-gAC 3'
Well No.	13	14	15

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²The nucleotide position 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 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*57:01 SSP typing kit																			
				Well ²															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
				Prod. No.:	201327601	201327602	201327603	201327604	201327605	201327606	201327607	201327608	201327609	201327610	201327611	201327612	201327613	201327614	201327615
IHWG cell line ¹		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 PTOU	*44:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*57:01		+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
13	9025 JESTHOM	*27:05		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLG	*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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

¹The provided cell line HLA specificities are retrieved from the <http://www.ihwg.org/hla> web site. The specificity of an individual cell line may thus be subject to change.

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²The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 3 to 5, 7 to 10 and 12 to 15 were available. The specificities of the primers in primer solutions 3 to 5, 7, 8, 10 and 12 to 15 were tested by separately adding additional 5'-primers, respectively 3'-primers. In primer solution 9 it was only possible to test the 3'-primer, the 5'-primers were not possible to test.

In primer solutions 3, 4, 6, 7, 10, 11, 12 and 15 one to three 3'-primers were not possible to test, and in primer solutions 4, 5, 8, 10, 14 and 15 one to three 5'-primers were not possible to test.

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Lot-specific information

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