

101.567-12 – including *Taq* pol., IFU-01
101.567-12u – without *Taq* pol., IFU-02

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

Lot No.: **94Y**

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

Product number:	101.567-12 – including <i>Taq</i> polymerase 101.567-12u – without <i>Taq</i> polymerase
Lot number:	94Y
Expiry date:	2018-April-01
Number of tests:	12
Number of wells per test:	30+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. 94Y.

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 LOT (28X)**

The HLA-B*57 kit is updated for new alleles to enable separation of:

- Confirmed¹ alleles as listed in the IMGT/HLA database
- Polymorphisms in exons outside of the region encoding the peptide binding domain
- Null and Alternatively expressed alleles

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

Three wells have been added to HLA-B*57, wells **29 to 31**.

¹As described in section Uniquely Identified Alleles.

The HLA-B*57 primer set, specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP®* HLA-B*57 lot was made (**Lot No. 28X**). The kit design is based on IMGT/HLA database 3.21.1.

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.

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

Well	5'-primer	3'-primer	rationale
18	Moved	Moved	Primer pair moved to well 28 for the allelic resolution of B*57:73 allele.
25	Modified	Exchanged	5'-primer modified and 3'-primer exchanged for decreased tendency of primer oligomer formation.
28	Added	Added	Negative Control moved to well 31, primer pair added from well 18 for the B*57:73 allele.
29	New	New	New primer pair for the B*57:78 allele.
30	New	New	New primer pair for the B*57:79N allele.
31	-	-	Negative control added from well 28.

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Well **31** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup SSP*® HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

HLA-specific PCR product sizes range from 75 to 200 base pairs.
The PCR product generated by the positive control primer pair is 430 base pairs.

Length of PCR product	105	200	105	80	75	80	85
5'-primer¹	164	340	440	45	45	43	36
	5'-CAC ^{3'}	5'-Agg ^{3'}	5'-TTA ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-TAC ^{3'}
							36
							5'-TAT ^{3'}
3'-primer²	231	2nd I	507	59	58	57	47
	5'-TgC ^{3'}	5'-AAA ^{3'}	5'-TTg ^{3'}	5'-CTC ^{3'}	5'-ggC ^{3'}	5'-CTC ^{3'}	5'-ACA ^{3'}
							48
							5'-gCA ^{3'}
							48
							5'-gCC ^{3'}
							52
							5'-TgT ^{3'}
A*	+	+	+				
B*	+	+	+				
C*	+	+	+				
DRB1				+	+		
DRB3				+	+		
DRB5				+			
DQB1					+		
DPB1						+	
DQA1							+

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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Lot-specific information

PRODUCT DESCRIPTION

HLA-B*57 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*57:01 to B*57:79N alleles.

PLATE LAYOUT

Each test consists of 31 PCR reactions in a 32 well cut PCR plate. Well 32 is empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	NC	empty

The 32 well cut PCR plate is marked with ‘HLA-B*57’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘94Y’.

Wells 1 to 30 – HLA-B*57 high resolution primers.

Well 31 – Negative Control (NC).

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 32 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 alleles will be amplified by primer mixes 1 to 6, 8, 9, 12 to 17, 19 to 22, 24, 26, 28 and 29. In addition, a few HLA-A and HLA-C alleles will be amplified by primer mixes 1, 2, 4, 5, 14, 15, 17, 19, 20, 25 and 26.

For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*57 alleles, i.e. **B*57:01 to B*57:79N**, recognized by the HLA Nomenclature Committee in August 2015^{1,2} will be amplified by the primers in the HLA-B*57 subtyping kit³.

The HLA-B*57 kit enables separation of the confirmed HLA-B*57 alleles as listed in the IMGT/HLA database. An HLA allele is listed as confirmed by IMGT/HLA if it has been sequenced by more than a single laboratory or from multiple sources. Current allele confirmation status for HLA-B*57 alleles is listed below.

The HLA-B*57 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.

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Lot-specific information

The following HLA-B57 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix
B*57:15, 57:37, 57:55	7
B*57:25, 57:41	19

The HLA-B*57 subtyping kit cannot distinguish the silent mutations in the B*57:01:01-57:01:04 and 57:01:06-57:01:22 alleles, the B*57:02:01-57:02:02 alleles, the B*57:03:01-57:03:02 alleles or the B*57:14:01-57:14:02 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2015-August-11 release 3.21.1, 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>.

³The B*57:45, 57:62 and 57:69 and the B*35:208 and B*58:14 alleles will give rise to identical amplification patterns. These alleles can e.g. be distinguished by the HLA-B low resolution kit and/or the HLA-B*35 and HLA-B*58 high resolution kit.

ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
B*57:01:01	Confirmed	B*57:08	Unconfirmed	B*57:37	Unconfirmed	B*57:67	Unconfirmed
B*57:01:02	Unconfirmed	B*57:09	Confirmed	B*57:38	Unconfirmed	B*57:68	Unconfirmed
B*57:01:03	Unconfirmed	B*57:10	Unconfirmed	B*57:39	Confirmed	B*57:69	Unconfirmed
B*57:01:04	Unconfirmed	B*57:11	Confirmed	B*57:40	Confirmed	B*57:70	Unconfirmed
B*57:01:05	Confirmed	B*57:12	Unconfirmed	B*57:41	Confirmed	B*57:71	Unconfirmed
B*57:01:06	Unconfirmed	B*57:13	Confirmed	B*57:42	Unconfirmed	B*57:72	Unconfirmed
B*57:01:07	Confirmed	B*57:14:01	Confirmed	B*57:43	Confirmed	B*57:73	Confirmed
B*57:01:08	Confirmed	B*57:14:02	Unconfirmed	B*57:44	Confirmed	B*57:74	Unconfirmed
B*57:01:09	Unconfirmed	B*57:15	Confirmed	B*57:45	Unconfirmed	B*57:75	Unconfirmed
B*57:01:10	Confirmed	B*57:16	Unconfirmed	B*57:46	Unconfirmed	B*57:76	Unconfirmed
B*57:01:11	Unconfirmed	B*57:17	Confirmed	B*57:47	Unconfirmed	B*57:77	Unconfirmed
B*57:01:12	Unconfirmed	B*57:18	Unconfirmed	B*57:48	Unconfirmed	B*57:78	Confirmed
B*57:01:13	Unconfirmed	B*57:19	Unconfirmed	B*57:49	Confirmed	B*57:79N	Unconfirmed
B*57:01:14	Confirmed	B*57:20	Confirmed	B*57:50	Unconfirmed		
B*57:01:15	Unconfirmed	B*57:21	Confirmed	B*57:51	Unconfirmed		
B*57:01:16	Unconfirmed	B*57:22	Confirmed	B*57:52	Unconfirmed		
B*57:01:17	Unconfirmed	B*57:23	Confirmed	B*57:53	Unconfirmed		
B*57:01:18	Unconfirmed	B*57:24	Confirmed	B*57:54	Unconfirmed		
B*57:01:19	Unconfirmed	B*57:25	Unconfirmed	B*57:55	Unconfirmed		
B*57:01:20	Unconfirmed	B*57:26	Confirmed	B*57:56	Unconfirmed		
B*57:01:21	Unconfirmed	B*57:27	Unconfirmed	B*57:57	Confirmed		
B*57:01:22	Unconfirmed	B*57:28N	Unconfirmed	B*57:58	Confirmed		
B*57:02:01	Confirmed	B*57:29	Confirmed	B*57:59	Unconfirmed		
B*57:02:02	Confirmed	B*57:30	Unconfirmed	B*57:60	Unconfirmed		
B*57:03:01	Confirmed	B*57:31	Unconfirmed	B*57:61	Confirmed		
B*57:03:02	Unconfirmed	B*57:32	Confirmed	B*57:62	Unconfirmed		
B*57:04	Confirmed	B*57:33	Unconfirmed	B*57:63	Confirmed		
B*57:05	Unconfirmed	B*57:34	Confirmed	B*57:64	Unconfirmed		
B*57:06	Unconfirmed	B*57:35	Confirmed	B*57:65	Unconfirmed		
B*57:07	Unconfirmed	B*57:36	Unconfirmed	B*57:66	Unconfirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2015-August-11, release 3.21.1, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-B*57 homo- and heterozygotes is available upon request.

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Lot-specific information
SPECIFICITY TABLE

HLA-B*57 SSP subtyping

Specificities and sizes of the PCR products of the 31+1 primer mixes used for HLA-B*57 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*57 alleles ³	Other amplified HLA Class I alleles ⁴
1	150 bp	800 bp	*57:01:01-57:01:04, 57:01:06-57:10, 57:12, 57:14:01-57:30, 57:32- 57:46, 57:48-57:73, 57:75-57:79N	*07:235, 08:49, 13:01:01-13:01:10, 13:06- 13:07N, 13:12:01-13:13:02, 13:17, 13:20, 13:22:01-13:23, 13:25-13:26:02, 13:28- 13:29, 13:36, 13:39, 13:43, 13:50-13:52, 13:57, 13:60-13:61, 13:63N, 13:73, 13:76N- 13:80, 13:83, 14:10, 15:02:01-15:02:09, 15:13:01-15:13:02, 15:20-15:21, 15:25:01- 15:25:03, 15:36, 15:44, 15:62, 15:77, 15:80, 15:85, 15:88-15:89, 15:106, 15:112, 15:121, 15:139, 15:144, 15:154, 15:165, 15:170, 15:194, 15:204, 15:213-15:214, 15:223, 15:240, 15:250, 15:265, 15:271, 15:283, 15:289, 15:291, 15:297, 15:301-15:302N, 15:308, 15:319, 15:325, 15:328, 15:330, 15:341, 15:345, 15:357-15:358, 18:22, 18:69, 18:105, 27:19, 27:30, 27:127, 35:01:01:01-35:04:03, 35:06-35:08:08, 35:10-35:17:02, 35:19-35:21, 35:23-35:30, 35:33-35:36, 35:38-35:42:02, 35:45-35:50, 35:52, 35:54-35:57, 35:59, 35:61:01-35:63, 35:65Q, 35:69-35:71, 35:74, 35:76-35:78, 35:80-35:85, 35:90-35:96, 35:98, 35:100- 35:101:02, 35:103-35:113, 35:115-35:116, 35:120-35:126, 35:128-35:134N, 35:136- 35:150:02, 35:152-35:173N, 35:175-35:184, 35:186-35:198, 35:200-35:204, 35:206- 35:229, 35:231, 35:233, 35:236-35:250, 35:252-35:259, 35:261-35:264, 35:266- 35:272, 35:274-35:275, 35:277-35:282, 35:285-35:291, 35:293-35:296, 37:01:01- 37:01:07, 37:01:09, 37:01:11, 37:03N- 37:06:02, 37:08, 37:10-37:11, 37:13-37:43, 37:45-37:50, 37:52-37:56, 38:20, 39:42, 40:28, 44:02:01:01-44:02:33, 44:02:35- 44:14, 44:16-44:17, 44:19N, 44:21-44:30, 44:32-44:40, 44:42-44:46, 44:48-44:52N, 44:55-44:64:02, 44:66-44:98, 44:101-44:105, 44:107-44:134, 44:136-44:137, 44:139- 44:157, 44:159-44:165, 44:167-44:181, 44:183, 44:185-44:191, 44:194-44:196, 44:198N-44:212, 44:214-44:223, 46:33, 48:02:01-48:02:03, 48:25, 50:17, 51:04, 51:42, 51:46, 51:56:01-51:56:03, 51:139, 53:01:01-53:13, 53:15-53:39, 55:14, 56:09,

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				56:11-56:12, 58:01:01:01-58:01:02, 58:01:04-58:01:15, 58:01:17-58:01:19, 58:04-58:05, 58:09-58:15, 58:17N, 58:19, 58:21-58:24, 58:28:01-58:29, 58:31N-58:37, 58:39N-58:42, 58:44-58:45:02, 58:47-58:59, 58:62-58:69, 58:72N, 83:01, C*03:102, C*03:263
2⁵	100 bp	1070 bp	*57:01:01-57:01:22, 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, 57:47-57:52, 57:54-57:56, 57:58-57:62, 57:64-57:65, 57:67-57:69, 57:71-57:75, 57:77-57:79N	*35:208, 55:14, 58:14, C*06:72
3	220 bp	1070 bp	*57:01:01-57:01:22, 57:03:01-57:03:02, 57:06-57:08, 57:10, 57:14:01-57:18, 57:20-57:23, 57:25-57:27, 57:29, 57:31-57:41, 57:43-57:51, 57:53-57:60, 57:62, 57:64-57:79N	*35:208, 40:30, 40:34, 44:153, 55:14, 58:14
4⁵	100 bp	800 bp	*57:02:01-57:03:02, 57:05, 57:07, 57:09, 57:12, 57:17, 57:39, 57:42, 57:46, 57:57, 57:63, 57:66, 57:70	*07:137, 08:60, 08:76 ^w , 08:129, 13:13:01-13:13:02, 13:21, 35:02:01-35:02:07, 35:04:01-35:04:03, 35:06 ^w , 35:09:01-35:09:03, 35:12:01-35:12:03, 35:18, 35:59 ^w , 35:81, 35:83, 35:88, 35:95, 35:129N, 35:149, 35:154, 35:157, 35:162, 35:172, 35:182-35:184, 35:201, 35:211, 35:220, 35:230, 35:233, 35:258, 35:266, 35:270, 35:285, 37:01:01 ^w -37:01:11 ^w , 37:03N ^w -37:06:02 ^w , 37:08 ^w , 37:10 ^w -37:11 ^w , 37:12, 37:13 ^w -37:18 ^w , 37:19, 37:20 ^w -37:24 ^w , 37:26 ^w -37:34 ^w , 37:36 ^w -37:43 ^w , 37:45 ^w -37:50 ^w , 37:52 ^w -37:55 ^w , 38:20 ^w , 39:42 ^w , 40:04, 40:28, 40:30, 40:34, 40:64, 40:68, 40:99 ^w , 40:129, 40:137, 40:160:01-40:160:02, 42:13, 44:62, 44:77, 44:82 ^w , 48:17 ^w , 51:04, 51:46, 51:56:01-51:56:03, 51:139, 53:19, 53:36, 56:12, 58:28:01-58:28:02, 58:65, C*05:10^w, C*15:24
5	220 bp	1070 bp	*57:02:01-57:02:02, 57:04, 57:12-57:13, 57:19, 57:28N, 57:30, 57:42, 57:63	*07:227, C*06:72
6⁵	95 bp 180 bp 205 bp	1070 bp	*57:04, 57:32 *57:06 *57:30	*44:153
7⁵	105 bp 170 bp 295 bp	1070 bp	*57:15 *57:37 *57:55	

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8 ⁵	90 bp 200 bp 250 bp	1070 bp	*57:16 *57:49 *57:07	*44:153
9 ⁶	170 bp	1070 bp	*57:09, 57:13, 57:22, 57:57, 57:63	*07:02:01-07:24, 07:26-07:47, 07:49N-07:50, 07:52-07:154, 07:156-07:163, 07:165- 07:201N, 07:203-07:208, 07:210-07:219, 07:221-07:222, 07:224-07:225, 07:227- 07:234, 07:236-07:249, 07:251N-07:256, 08:20, 08:53:01-08:53:02, 08:79, 13:16, 13:20, 13:31, 13:48, 13:62, 14:01:01-14:40, 14:42, 14:44-14:49, 15:01:01:01-15:01:04, 15:01:06-15:04:02, 15:06-15:19, 15:21, 15:23-15:30, 15:32:01-15:40, 15:42- 15:47:02, 15:49-15:50, 15:53-15:54, 15:56- 15:58, 15:60-15:74, 15:76-15:82, 15:85, 15:87, 15:89-15:90, 15:92-15:99, 15:101- 15:104, 15:106, 15:108-15:110, 15:112- 15:113, 15:115-15:122, 15:125-15:129, 15:131-15:135, 15:137-15:144, 15:146- 15:150, 15:152-15:154, 15:156-15:161, 15:163-15:175, 15:177-15:178, 15:180- 15:184, 15:187, 15:189-15:199, 15:201- 15:217, 15:219-15:221, 15:223, 15:225- 15:234, 15:236, 15:238-15:249, 15:251- 15:274, 15:276-15:282, 15:284-15:311, 15:313-15:331, 15:333-15:347, 15:353- 15:363, 18:15, 18:19, 18:21, 18:30, 18:57, 18:101, 27:04:01-27:04:04, 27:06, 27:10, 27:15, 27:18, 27:20-27:21, 27:24-27:25, 27:40, 27:54, 27:63, 27:66N, 27:68-27:69, 27:79, 27:86, 27:91-27:92, 27:100, 27:103, 27:105-27:109, 27:112-27:115, 27:120, 27:138, 35:11:01-35:11:03, 35:14:01- 35:14:02, 35:21, 35:43:01-35:44, 35:58, 35:67, 35:79, 35:86, 35:96, 35:99, 35:102, 35:117-35:118, 35:135, 35:167, 35:185, 35:188, 35:213, 35:232, 35:265, 35:277, 37:07, 37:13, 38:10, 38:32, 39:18, 39:35- 39:36, 40:05, 40:15-40:16, 40:23, 40:26, 40:28, 40:32, 40:51, 40:95, 40:98, 40:148, 40:158, 40:161, 40:174, 40:183, 40:198, 40:309, 42:19, 44:76, 44:79:01-44:79:02, 44:146, 44:150, 46:01:01-46:05, 46:07N- 46:08, 46:10, 46:12, 46:14-46:17, 46:20, 46:22-46:24, 46:26-46:32, 46:34-46:42, 46:44-46:64, 47:10, 48:05, 48:08, 48:15, 48:25, 49:01:01-49:10, 49:12-49:14, 49:16- 49:39, 50:01:01-50:02, 50:04-50:20, 50:31- 50:43, 51:01:01:01-51:04, 51:06:01- 51:07:02, 51:11N-51:14, 51:16-51:18, 51:21- 51:24:05, 51:26-51:30, 51:32-51:39, 51:41N, 51:43, 51:45-51:46, 51:48-51:52, 51:55- 51:72, 51:74-51:80, 51:82-51:92:02, 51:94- 51:96, 51:98N-51:107, 51:109-51:114, 51:116-51:119, 51:121, 51:123-51:138,

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10	210 bp	1070 bp	*57:08	
11	215 bp 250 bp	800 bp	*57:09, 57:24 *57:14:01-57:14:02, 57:43	
12⁵	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, 57:66, 57:70	*40:30, 40:34
13⁵	90 bp	800 bp	*57:01:01-57:15, 57:17-57:19, 57:21- 57:35, 57:37-57:44, 57:46-57:50, 57:52- 57:61, 57:63-57:68, 57:70-57:79N	*58:36
14	135 bp 195 bp	1070 bp	*57:17 *57:10	*44:189, C*07:239 *07:219
15^{5,7}	110 bp 145 bp	1070 bp	*57:29, 57:33 *57:11	*07:120, 15:214, 18:81, 35:250, 40:150, 51:165 *14:01:01-14:02:08, 14:02:10-14:04, 14:07N, 14:09, 14:11-14:12, 14:14-14:36, 14:38- 14:49, 18:44:01-18:44:02, 39:79, 58:02, 58:06-58:07, 58:25, 58:38, 58:43, 58:60, C*01:32, C*06:20
16⁵	85 bp 160 bp	1070 bp	*57:12 *57:18	*08:119, 14:24, 35:226, 39:92, 58:64
17	140 bp 210 bp	1070 bp	*57:13, 57:31 *57:13, 57:22, 57:27, 57:57, 57:63	*07:227, 40:30, 40:34, C*06:72 *07:227, 55:14, C*06:72^w
18	165 bp	1070 bp	*57:23	
19⁵	90 bp 240 bp	1070 bp	*57:04, 57:41 *57:13, 57:25, 57:43	*44:153, C*06:72 *07:227, 40:30, 40:34
20⁵	90 bp 240 bp	1070 bp	*57:20 *57:26	*55:14, C*06:72
21⁵	110 bp 150 bp	1070 bp	*57:33 *57:21	*35:127
22⁵	95 bp 150 bp	800 bp	*57:34 *57:40	*58:54 *14:20

101.567-12 – including *Taq* pol., IFU-01
101.567-12u – without *Taq* pol., IFU-02

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“Instructions for Use” (IFU)

Lot No.: **94Y**

Lot-specific information

23 ⁵	75 bp 100 bp	1070 bp	*57:35 *57:36	
24	150 bp	1070 bp	*57:40	*14:20
25	235 bp	1070 bp	*57:44	A*32:29
26	155 bp	1070 bp	*57:58	*07:219, 58:12, A*02:42, A*02:310
27	235 bp	1070 bp	*57:61	
28	200 bp	1070 bp	*57:39, 57:51, 57:73	*58:41
29	150 bp	1070 bp	*57:78	*15:340, 40:218, 46:28, 51:58
30	175 bp	1070 bp	*57:79N	
31 ⁸	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 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.

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. Assumptions 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 alleles will be amplified by primer mixes 1 to 6, 8, 9, 12 to 17, 19 to 22, 24, 26, 28 and 29. In addition, a few HLA-A and HLA-C alleles will be amplified by primer mixes 1, 2, 4, 5, 14, 15, 17, 19, 20, 25 and 26.

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

⁶Primer mix 9 may have tendencies of unspecific amplifications.

⁷Primer mix 15 has a tendency to giving rise to primer oligomer formation.

⁸Primer mix 31 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by the control primer pairs matching the human growth hormone gene. HLA-specific PCR product sizes range from 75 to 200 base pairs and the PCR product generated by the HGH positive control primer pair is 430 base pairs.

'w', might be weakly amplified.

101.567-12 – including *Taq* pol., IFU-01
101.567-12u – without *Taq* pol., IFU-02

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Lot No.: **94Y**

Lot-specific information
PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	150	100	220	100	220	95	105	90	170	210	215	90
						180	170	200			250	
						205	295	250				
Length of int. pos. control ¹	800	1070	1070	800	1070	1070	1070	1070	1070	1070	800	1070
5'-primer(s) ²	355 5'-TCA 3'	362 5'-ggT 3'	362 5'-ggT 3'	355 5'-TCA 3'	362 5'-ggT 3'	362 5'-ggT 3'	209 5'-ggC 3'	97 5'-TCg 3'	527 5'-TgA 3'	320 5'-CCC 3'	362 5'-ggT 3'	362 5'-ggT 3'
							757 5'-CCA 3'	209 5'-ggA 3'				
							878 5'-gCA 3'	362 5'-ggT 3'				
3'-primer(s) ³	463 5'-gCT 3'	419 5'-Cgg 3'	539 5'-TCA 3'	412 5'-gTT 3'	539 5'-TCC 3'	418 5'-gTC 3'	271 5'-CAC 3'	256 5'-CCC 3'	3 rd 5'-TAT 3'	2 nd 5'-TCg 3'	538 5'-gTC 3'	412 5'-gTT 3'
		419 5'-CAg 3'				500 5'-ggA 3'	916 5'-gAC 3'	572 5'-gCg 3'			559 5'-Cgg 3'	
						527 5'-CCg 3'					583 5'-gTg 3'	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15	16	17	18	19	20	21	22	23	24
Length of spec. PCR product	90	135	110	85	140	165	90	90	110	95	75	150
		195	145	160	210		240	240	150	150	100	
Length of int. pos. control ¹	800	1070	1070	1070	1070	1070	1070	1070	1070	800	1070	1070
5'-primer(s) ²	209 5'-ggC 3'	103 5'-CCT 3'	361 5'-AgT 3'	256 5'-ACg 3'	362 5'-ggT 3'	130 5'-AgT 3'	362 5'-ggT 3'	209 5'-ggC 3'	352 5'-ACg 3'	200 5'-TCg 3'	209 5'-ggC 3'	353 5'-CAA 3'
		446 5'-CgA 3'	392 5'-CgA 3'	362 5'-ggT 3'				362 5'-ggT 3'	392 5'-CgA 3'	353 5'-CAA 3'		
			704 5'-TgT 3'									
3'-primer(s) ³	256 5'-CCC 3'	256 5'-CCC 3'	463 5'-gCT 3'	302 5'-ggC 3'	463 5'-gCg 3'	256 5'-CCC 3'	409 5'-ATA 3'	259 5'-CTT 3'	463 5'-gCT 3'	256 5'-CCC 3'	244 5'-CTT 3'	463 5'-gCT 3'
		539 5'-TCA 3'	774 5'-ggT 3'	481 5'-gTA 3'	527 5'-CCT 3'		559 5'-CTC 3'	559 5'-CgT 3'		463 5'-gCT 3'	268 5'-gTg 3'	
					537 5'-Agg 3'		559 5'-Cgg 3'					
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

Well No.	25	26	27	28	29	30
Length of spec. PCR product	235	155	235	200	150	175
Length of int. pos. control ¹	1070	1070	1070	1070	1070	1070
5'-primer(s) ²	122 5'-CCT 3'	142 5'-TCT 3'	362 5'-ggT 3'	97 5'-TCC 3'	106 5'-CCA 3'	877 5'-AC 3'
3'-primer(s) ³	317 5'-ggA 3'	256 5'-CCC 3'	554 5'-CCC 3'	256 5'-CCC 3'	214 5'-CCA 3'	916 5'-gAC 3'
Well No.	25	26	27	28	29	30

101.567-12 – including *Taq* pol., IFU-01
101.567-12u – without *Taq* pol., IFU-02

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Lot No.: 94Y

Lot-specific information

¹The 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.

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.

101.567-12 – including *Taq* pol., IFU-01
101.567-12u – without *Taq* pol., IFU-02

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Lot No.: **94Y**

Lot-specific information

CELL LINE VALIDATION SHEET																			
HLA-B*57 SSP subtyping kit²																			
			Prod. No.:	Well															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				201326401	201326402	201326403	201326404	201326405	201326406	201326407	201326408	201326409	201326410	201441111	201326412	201326413	201326414	201326415	201326416
	IHWC cell line¹	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 OLGGA	*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	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

101.567-12 – including *Taq* pol., IFU-01
101.567-12u – without *Taq* pol., IFU-02

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Lot No.: **94Y**

Lot-specific information

CELL LINE VALIDATION SHEET			Well													
HLA-B*57 SSP subtyping kit ²			17	18	19	20	21	22	23	24	25	26	27	28	29	30
		Prod. No.:	201326417	201557718	201326419	201557720	201326421	201326422	201326423	201441124	201557725	201441126	201441127	201557728	201557729	201557730
	IHWC 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 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	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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.

101.567-12 – including *Taq* pol., IFU-01
101.567-12u – without *Taq* pol., IFU-02

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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 6 to 8, 11, 14 and 16 to 30 were available. The specificities of the primers in primer solutions 6 to 8, 11, 14, 16, 17, 19, 20, 26 and 28 were tested by separately adding additional 5'-primers, respectively additional 3'-primers. In primer solutions 23, 27 and 29 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solution 18, 21, 22, 24, 25 and 30 it was only possible to test the 3'-primer, the 5'-primer was not possible to test.

In primer solutions 2, 6, 7, 11, 16, 17, 19 and 20 one of the 3'-primers was not possible to test, and in primer solutions 7, 8, 14 and 15 one or two of the 5'-primers were not possible to test. One additional 3'-primer in primer solution 15 was tested by separately adding one 5'-primer.

101.567-12 – including *Taq* pol., IFU-01
101.567-12u – without *Taq* pol., IFU-02

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Lot-specific information

ADDRESSES:

Manufacturer:

Olerup SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.

Tel: +46-8-717 88 27

Fax: +46-8-717 88 18

E-mail: info-ssp@olerup.com

Web page: <http://www.olerup-ssp.com>

Distributed by:

Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.

Tel: +43-1-710 15 00

Fax: +43-1-710 15 00 10

E-mail: support-at@olerup.com

Web page: <http://www.olerup.com>

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382

Tel: 1-877-OLERUP1

Fax: 610-344-7989

E-mail: info.us@olerup.com

Web page: <http://www.olerup.com>

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.