

Olerup SSP[®] HLA-B*56

Product number:	101.571-06 – including <i>Taq</i> polymerase
Lot number:	35K
Expiry date:	2012-September-01
Number of tests:	6
Number of wells per test:	24
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. 35K.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] HLA-B*56 LOT

The HLA-B*56 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP*[®] HLA-B*56 lot was made (Lot No. 70F).

The amplification patterns for some rare HLA-B*56 alleles only differ by the length of the specific PCR products.

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	-	Added	Primer added for the B*56:01:04 allele.
7	-	Added	Primer added for the B*56:25 allele, increased yield of specific PCR product.
15	Added	Added	Primer pair added for the B*56:28N allele
19	Added	Added	Primer pair added for the B*56:29 allele
21	-	Modified, added	Primer added for the B*56:26 allele, improved specificity.
24	Exchanged	Exchanged	Changed to more specific primer pair.

Change in revision R01 compared to R00:

1. The B*56:06 allele is not amplified by primer mix 5. This has been revised in the specificity and interpretation tables.

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General “Instructions for Use”

IFU-01 Rev. No. 03 can be downloaded from

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PRODUCT DESCRIPTION

HLA-B*56 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*56:01 to B*56:29 alleles.

PLATE LAYOUT

Each test consists of 24 PCR reactions in a 24 well PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24

The 24 well PCR plate is marked with ‘HLA-B*56’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘35K’.

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 covered with a PCR-compatible foil.

Please note: When removing each 24 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

The interpretation of HLA-B*56 SSP subtypings will be influenced by nine B*07, seven B*08, most B*13, two B*14, several B*15, most B*18, four B*27, most B*35, most B*37, nine B*38, seven B*39, thirteen B*40, the B*42:09, most B*44, the B*45, four B*46, three B*48, the B*49, the B*50, the B*51, the B*52, the B*53, the B*54, the B*55, most B*57, the B*58, the B*59, the B*78, three B*81, the B*82 and the B*83:01 alleles when present on the other haplotype. In addition, the C*02:06 and C*12:15 alleles will be amplified by primer mix 4, the C*03:12, C*03:19 and 15:26 alleles weakly by primer mix 14, the C*03:81 by primer mix 17, the C*06:20 and C*07:81 by primer mix 1 and the C*07:123, C*15:09, C*15:19, C*15:20, C*15:27 and C*16:20 alleles by primer mix 22.

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UNIQUELY IDENTIFIED ALLELES

All the HLA-B*56 alleles, i.e. **B*56:01 to B*56:29**, recognized by the HLA Nomenclature Committee in July 2010¹ will give rise to unique amplification patterns by the primers in the HLA-B*56 subtyping kit².

The HLA-B*56 subtyping kit cannot distinguish the B*56:01:01 to 56:01:04 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2010-January-16, release 3.1.0, www.ebi.ac.uk/imgt/hla.

²The HLA-B*56 primer set cannot separate the B*56:05:02 and B*56:06 alleles. These two alleles can be distinguished by the HLA-B low resolution kit.

The HLA-B*56 primer set cannot separate the B*56:09, B*35:76 and B*35:78 alleles. These alleles can be distinguished by the HLA-B low resolution and/or B*35 kits.

The B*56:13 and 56:28N alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 15.

The B*56:17 and 56:26 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 21.

The B*56:19N and 56:29 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 19.

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 33 alleles generate 27 amplification patterns that can be combined in 378 homozygous and heterozygous combinations. 152 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.

++++---+ ---+-----	-----	*56:08, *56:10 = *56:10, *56:14
++++---+ ---+---+--	-----	*56:04, *56:08 = *56:04, *56:14
++++---+ ---+-----	-----	*56:02, *56:08 = *56:02, *56:14
++++---+ ---+-----	-----	*56:02, *56:09 = *56:02, *56:12
++++---+ ---+-----	-----+---	*56:02, *56:23 = *56:10, *56:17
++++---+ ---+-----	-----+--	*56:10, *56:20 = *56:10, *56:24
++++---+ ---+---+--	-----	*56:01:01, *56:04 = *56:02, *56:27
++++---+ ---+-----	-----+--	*56:02, *56:20 = *56:02, *56:24
+++-+---+ ---+-----	+-----	*56:06, *56:08 = *56:06, *56:14
+++-+---+ ---+-----	+-----	*56:01:01, *56:06 = *56:06, *56:25
+++-+---+ ---+-----	-----	*56:03, *56:08 = *56:03, *56:14
+++-+---+ ---+-----	+-----	*56:05:01, *56:08 = *56:05:01, *56:14
+++-+---+ ---+-----	+-----	*56:01:01, *56:05:01 = *56:05:01, *56:25
+++-+---+ ---+-----	+-----+	*56:08, *56:21 = *56:14, *56:21
+++-+---+ ---+-----	+-----	*56:05:02, *56:08 = *56:05:02, *56:14
+++-+---+ ---+-----	-----	*56:08, *56:25 = *56:14, *56:25
+++-+---+ ---+-----	-----	*56:09, *56:25 = *56:12, *56:25
+++-+---+ ---+-----	+-----+	*56:01:01, *56:21 = *56:21, *56:25
+++-+---+ ---+-----	+-----	*56:01:01, *56:05:02 = *56:05:02, *56:25 = *56:15, *56:25
+++-+---+ ---+-----	-----+--	*56:20, *56:25 = *56:24, *56:25
+++-+---+ ---+-----	-----	*56:01:01, *56:25 = *56:25, *56:25
+++-+---+ ---+-----	-----	*56:07, *56:08 = *56:07, *56:14
+++-+---+ ---+---+--	-----	*56:08, *56:11 = *56:11, *56:14
+++-+---+ ---+-----	-----	*56:08, *56:09 = *56:08, *56:12 = *56:09, *56:14 = *56:12, *56:14
+++-+---+ ---+-----	-----+---	*56:08, *56:23 = *56:14, *56:23
+++-+---+ ---+---+--	-----	*56:08, *56:22 = *56:14, *56:22
+++-+---+ ---+---+--	-----	*56:08, *56:27 = *56:14, *56:27
+++-+---+ ---+-----	-----	*56:08, *56:13 = *56:13, *56:14

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+++-----+ --+----- +-----	*56:08, *56:15 = *56:14, *56:15
+++-----+ --+----- -+-----	*56:08, *56:16 = *56:14, *56:16
+++-----+ --+----- --+-----	*56:08, *56:19N = *56:14, *56:19N
+++-----+ --+----- -----+---	*56:08, *56:17 = *56:14, *56:17
+++-----+ --+----- -----+---	*56:08, *56:18 = *56:14, *56:18
+++-----+ --+----- -----+---	*56:08, *56:20 = *56:08, *56:24 = *56:14, *56:20 = *56:14, *56:24
+++-----+ --+----- -----	*56:01:01, *56:08 = *56:01:01, *56:14 = *56:08, *56:08 = *56:08, *56:14
+++----- +++----- -----	*56:07, *56:09 = *56:07, *56:12
+++----- ++----- -----+---	*56:07, *56:20 = *56:07, *56:24
+++----- ++----- -----	*56:01:01, *56:07 = *56:07, *56:07
+++----- ++----- -----+---	*56:11, *56:20 = *56:11, *56:24
+++----- ++----- -----	*56:09, *56:13 = *56:12, *56:13
+++----- ++----- -+-----	*56:09, *56:16 = *56:12, *56:16
+++----- ++----- --+-----	*56:09, *56:19N = *56:12, *56:19N
+++----- ++----- -----+---	*56:09, *56:17 = *56:12, *56:17
+++----- ++----- -----+---	*56:09, *56:20 = *56:09, *56:24 = *56:12, *56:24
+++----- ++----- -----	*56:01:01, *56:09 = *56:01:01, *56:12
+++----- ++----- -----+---	*56:20, *56:23 = *56:23, *56:24
+++----- ++----- -----	*56:01:01, *56:23 = *56:17, *56:23
+++----- ++----- -----+---	*56:20, *56:22 = *56:22, *56:24
+++----- ++----- -----+---	*56:13, *56:20 = *56:13, *56:24
+++----- ++----- -----	*56:01:01, *56:13 = *56:13, *56:13
+++----- ++----- +-----+---	*56:15, *56:20 = *56:15, *56:24
+++----- ++----- -+-----+---	*56:16, *56:20 = *56:16, *56:24
+++----- ++----- -+-----	*56:01:01, *56:16 = *56:16, *56:16
+++----- ++----- --+-----+---	*56:19N, *56:20 = *56:19N, *56:24
+++----- ++----- --+-----	*56:01:01, *56:19N = *56:19N, *56:19N
+++----- ++----- -----+---	*56:17, *56:20 = *56:17, *56:24
+++----- ++----- -----+---	*56:01:01, *56:17 = *56:17, *56:17
+++----- ++----- -----+---	*56:01:01, *56:20 = *56:01:01, *56:24 = *56:20, *56:24 = *56:24, *56:24
++-+----- -+----- -----	*56:09, *56:10 = *56:10, *56:12
++----- +++----- -----+---	*56:09, *56:23 = *56:12, *56:23
++----- -+-----+-----	*56:09, *56:11 = *56:09, *56:22 = *56:11, *56:11 = *56:11, *56:12 = *56:11, *56:22 = *56:12, *56:22
++----- -+----- +-----	*56:09, *56:15 = *56:12, *56:15
++----- -+----- -----	*56:09, *56:09 = *56:09, *56:12
-+-----+++----- +-----	*56:03, *56:05:01 = *56:05:01, *56:06
-+-----+----- +-----+---	*56:03, *56:21 = *56:06, *56:21
-+-----+----- +-----	*56:03, *56:05:02 = *56:03, *56:06 = *56:05:02, *56:06 = *56:06, *56:06
-+-----+++----- +-----	*56:05:01, *56:05:01 = *56:05:01, *56:05:02
-+-----+----- +-----+---	*56:05:02, *56:21 = *56:21, *56:21

56:01:01 = 56:01:01-56:01:04

56:13 = 56:13 and 56:28N

56:17 = 56:17 and 56:26

56:19N = 56:19N and 56:29

SPECIFICITY TABLE

HLA-B*56 SSP subtyping

Specificities and sizes of the PCR products of the 24 primer mixes used for HLA-B*56 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-B*56 alleles ³	Other amplified HLA Class I alleles ⁴
1⁵	125 bp	800 bp	*56:01:01-56:02, 56:07-56:11, 56:13-56:17, 56:19N, 56:22-56:26, 56:28N-56:29	*13:01:01-13:04, 13:06-13:09, 13:11-13:20, 13:22:01-13:23, 13:25-13:34, 13:36-13:39, 15:16, 15:20, 15:34, 15:42, 15:62, 15:67, 15:85-15:86, 15:95, 15:137, 15:194, 18:22, 27:14, 35:01:01:01-35:08:04, 35:10-35:11:02, 35:13-35:15, 35:19-35:21, 35:23-35:30, 35:33-35:38, 35:40N-35:42:02, 35:45-35:50, 35:52, 35:54-35:58, 35:60, 35:62-35:63, 35:65Q-35:66, 35:68:01-35:72, 35:74-35:78, 35:80-35:85, 35:89-35:98, 35:100-35:101:02, 35:103-35:112, 35:115-35:116, 35:119-35:124, 35:126-35:134N, 35:136-35:145N, 40:28, 44:10, 44:15, 44:18, 44:48, 45:01-45:12, 46:11, 46:18, 48:02:01-48:02:02, 49:01:01-49:10, 50:01:01, 50:02, 50:05-50:09, 51:15, 51:56, 53:01:01-53:06, 53:08:01-53:23, 54:01-54:05N, 54:07-54:08N, 54:10, 54:12-54:23, 55:01:01-55:01:05, 55:02:01-55:02:03, 55:02:05-55:03, 55:05, 55:07, 55:09-55:13, 55:15-55:16, 55:18-55:19, 55:21-55:37, 55:39-55:43, 57:05, 58:01:01-58:01:02, 58:01:04-58:02, 58:04-58:07, 58:09, 58:11-58:19, 58:21-58:26, 58:28-58:30, 59:01, 59:03-59:05, 82:01-82:03, C*06:20, C*07:81
2	400 bp	1070 bp	*56:01:01-56:13, 56:15-56:29	*07:65, 35:76, 35:78, 44:90, 54:01-54:06, 54:08N-54:23, 55:01:01-55:05, 55:07-55:17, 55:19-55:33, 55:35-55:43, 81:01, 81:03 ² -81:04N ² , 82:01-82:03, 83:01

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3	180 bp	1070 bp	*56:01:01- 56:02, 56:04, 56:07-56:08, 56:13-56:14, 56:16-56:17, 56:19N-56:20, 56:24-56:29	*13:03, 15:73, 40:71, 44:10, 44:15, 44:18, 45:01, 45:04-45:07, 45:11- 45:12, 46:11, 49:01:01-49:03, 49:06, 49:08-49:10, 50:01:01-50:02, 50:04- 50:08, 51:15, 51:62, 54:03, 59:04, 82:01-82:03
4⁵	105 bp	800 bp	*56:02, 56:04, 56:10	*13:11, 15:18:03, 15:73, 15:86, 39:17, 40:48, 40:71, 51:62, 82:01-82:03, C*02:06, C*12:15
5	285 bp	1070 bp	*56:03	*55:35
6⁵	105 bp	1070 bp	*56:05:01	
7^{5,6,8}	120 bp, 210 bp	800 bp	*56:05:01- 56:06, 56:21, 56:25	*07:78 ^w , 07:84 ^w , 13:16, 15:16, 15:42, 15:67, 15:95, 40:95, 49:01:01, 49:02- 49:10, 50:01:01-50:02, 50:04-50:09, 51:01:01-51:03, 51:05, 51:07:01- 51:09:02, 51:11N-51:14, 51:16- 51:24:03, 51:26-51:41N, 51:43- 51:44N, 51:48-51:55, 51:57-51:58, 51:60-51:61, 51:63, 51:65-51:80, 51:82-51:96, 52:01:01-52:21, 54:20 ^w , 55:01:01-55:01:06, 55:03, 55:05, 55:09 ^w , 55:11, 55:15, 55:17, 55:24- 55:25, 55:28-55:29, 55:31, 55:33, 55:36, 55:38, 55:40, 58:08, 78:01- 78:07
8	395 bp	1070 bp	*56:08, 56:14	
9	145 bp	1070 bp	*56:07	*08:02, 15:115, 27:01, 38:02:01- 38:04, 38:08, 38:15, 38:18, 38:23, 44:40, 44:44
10⁷	150 bp	800 bp	*56:09, 56:11- 56:12	*08:49, 13:01:01-13:01:05, 13:06- 13:07N, 13:12-13:13, 13:17, 13:20, 13:22:01-13:23, 13:25-13:26, 13:28- 13:29, 13:36, 13:39, 14:10, 15:02:01- 15:02:04, 15:13, 15:20-15:21, 15:25:01-15:25:02, 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, 18:22, 27:19, 27:30, 35:01:01:01-35:04:03, 35:06- 35:08:04, 35:10-35:17, 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, 35:61-35:63, 35:65Q, 35:69-35:71, 35:74, 35:76-35:78,

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				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, 35:136-35:145N, 37:01:01-37:01:07, 37:03N-37:06, 37:08, 37:10-37:11, 37:13-37:23, 38:20, 39:42, 40:28, 44:02:01:01-44:14, 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, 44:107-44:110, 48:02:01-48:02:02, 51:04, 51:42, 51:46, 51:56, 53:01:01-53:13, 53:15-53:23, 55:14, 57:01:01-57:01:04, 57:01:06-57:10, 57:12, 57:14-57:20, 57:22-57:30, 57:32, 58:01:01-58:01:02, 58:01:04-58:01:07, 58:04-58:05, 58:09-58:15, 58:17N, 58:19, 58:21-58:24, 58:28-58:30, 83:01
11⁵	105 bp	800 bp	*56:01:01-56:01:04, 56:07-56:08, 56:13-56:14, 56:16-56:17, 56:19N-56:20, 56:23-56:29	*07:78, 13:02:01-13:03, 13:08Q-13:09, 13:14-13:16, 13:18-13:19, 13:27, 13:30-13:34, 13:37-13:38, 15:42, 35:60, 44:15, 44:18, 45:01, 45:03-45:08, 45:10-45:12, 46:11, 46:18, 49:01:01-49:03, 49:06-49:10, 50:01:01-50:02, 50:04-50:08, 51:15, 54:01-54:03, 54:05N, 54:07-54:08N, 54:10, 54:12-54:13, 54:16-54:23, 55:01:01-55:01:03, 55:01:05-55:03, 55:05, 55:07, 55:09-55:12, 55:15-55:16, 55:18-55:19, 55:21-55:22, 55:24-55:26, 55:29-55:31, 55:33-55:38, 55:40-55:41, 55:43, 59:01, 59:04-59:05
12⁷	180 bp	800 bp	*56:10, 56:23	*13:06, 15:42, 15:86, 35:60, 39:17 ^w , 45:08 ^w , 46:18, 54:01-54:02, 54:05N, 54:07-54:08N, 54:10, 54:12-54:13, 54:17-54:23, 55:01:01-55:03, 55:05, 55:07, 55:10-55:12, 55:15-55:16, 55:18-55:19, 55:21, 55:25-55:26, 55:29-55:31, 55:33-55:43, 59:01, 59:05
13⁷	180 bp	1070 bp	*56:11, 56:22	*15:58, 15:133, 35:03:01-35:03:04, 35:06, 35:13, 35:36, 35:38, 35:55-35:56, 35:59, 35:70, 35:74-35:75, 35:84-35:85, 35:98, 35:106, 35:109, 35:127-35:128, 35:136, 39:32-39:33,

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				51:13:01-51:13:02, 51:92, 52:14, 53:04
14⁵	80 bp	1070 bp	*56:04, 56:27	*15:18:03, 15:73, 39:17, 40:48, 40:71, 50:04, 51:62, 55:38, C*03:12^w, C*03:19^w, C*15:26^w
15⁹	160 bp, 380 bp	1070 bp	*56:13, 56:28N	*44:15, 44:18, 45:01, 45:04-45:08, 45:10-45:12, 82:01-82:03
16	160 bp	1070 bp		*44:02:01:01-44:02:13, 44:06, 44:08-44:09, 44:11-44:12, 44:16, 44:19N-44:24, 44:27, 44:33-44:34, 44:41, 44:44, 44:48-44:49, 44:52N-44:53, 44:55, 44:59, 44:63, 44:66-44:68, 44:71-44:74, 44:80, 44:83-44:84, 44:86-44:87, 44:89-44:91, 44:93, 44:95, 44:97, 44:99-44:102, 44:104, 44:106, 83:01
17⁷	180 bp	800 bp	*56:05:01-56:06, 56:15, 56:21	*07:20, 07:24, 07:60, 07:100, 08:21, 08:25, 15:09-15:10:02, 15:30, 15:37, 15:45, 15:48, 15:63, 15:90, 15:99, 15:150, 35:02:01-35:02:04, 35:04:01-35:04:03, 35:09:01-35:09:02, 35:12:01-35:12:02, 35:18, 35:22, 35:34, 35:39, 35:44, 35:81, 35:83, 35:95, 35:129N, 40:05, 40:26, 40:28, 40:63, 40:92, 44:62, 44:77, 44:82, 44:107, 51:01:01-51:01:14, 51:01:16-51:09:02, 51:11N-51:12, 51:18-51:20, 51:22-51:24:03, 51:26-51:30, 51:32-51:33, 51:35, 51:38-51:41N, 51:43-51:44N, 51:46, 51:48-51:61, 51:64-51:80, 51:83-51:84, 51:86-51:91, 51:94-51:96, 52:01:01-52:07, 52:09-52:13, 52:15-52:21, 53:19, 55:08, 57:02:01-57:03:02, 57:05, 57:07, 57:09, 57:12, 57:17, 57:28N, 58:08, 58:28, 78:01-78:06, C*03:81
18⁶	295 bp	800 bp	*56:16	*54:21, 55:10, 82:01-82:03
19^{5,10}	75 bp, 185 bp	800 bp	*56:19N, 56:29	*14:07N, 39:40N
20	215 bp	1070 bp		*07:02:19, 15:01:04, 37:01:04, 40:94, 54:01-54:23
21^{5,11}	110 bp, 170 bp	1070 bp	*56:17, 56:23, 56:26	*07:37
22	180 bp	1070 bp	*56:18	*08:37, 15:50, 15:69, 15:93, 15:186, 15:199, 18:01:01-18:09, 18:11-18:15,

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				18:17N-18:19, 18:22-18:25, 18:27-18:34, 18:37-18:40, 18:42-18:49, 35:35, 35:93, 39:07, 39:43, 42:09, 46:03, 46:21, 54:09, 54:14, 55:14, 55:23, 57:26, 58:05, C*07:123, C*15:09, C*15:19-15:20, C*15:27, C*16:20
23^{5,12}	85 bp, 350 bp	1070 bp	*56:20, 56:24	*07:78, 40:48
24	380 bp	1070 bp	*56:21	*08:03, 08:52, 40:13, 40:19, 40:109, 40:117, 48:18, 51:01:01-51:01:07, 51:01:09-51:01:14, 51:01:16-51:12, 51:16-51:24:03, 51:26-51:36, 51:38-51:41N, 51:43-51:44N, 51:46, 51:48-51:49, 51:51-51:53, 51:55-51:61, 51:64-51:77, 51:79-51:84, 51:86-51:89, 51:91, 51:93-51:96, 52:01:01-52:11, 52:13, 52:15-52:19, 52:21, 53:19, 57:02:01-57:03:02, 57:05, 57:07, 57:09, 57:17, 58:08, 58:27-58:28, 59:02

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*56 high resolution 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 1 070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*56 subtyping.

In addition, wells number 4, 7, 10 to 12 and 17 to 19 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.

³For several HLA-B alleles first exon 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. We assume that unknown sequences in the first exon are conserved within allelic groups.

The HLA-B*56 primer set cannot separate the B*56:09, B*35:76 and B*35:78 alleles. These alleles can be distinguished by the HLA-B low resolution and/or B*35 kits.

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The B*56:13 and 56:28N alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 15.

The B*56:17 and 56:26 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 21.

The B*56:19N and 56:29 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 19.

⁴Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B*56 alleles will be amplified by primer mixes 1 to 5, 7 and 9 to 24. In addition, the C*02:06 and C*12:15 alleles will be amplified by primer mix 4, the C*03:12, C*03:19 and 15:26 alleles weakly by primer mix 14, the C*03:81 by primer mix 17, the C*06:20 and C*07:81 by primer mix 1 and the C*07:123, C*15:09, C*15:19, C*15:20, C*15:27 and C*16:20 alleles by primer mix 22.

⁵Specific PCR fragments shorter than 125 base pairs have a lower intensity than longer PCR bands.

⁶Primer mix 7 and 18 may give rise to less specific PCR products than the other HLA-B*56 primer mixes.

⁷Primer mixes 10, 12, 13 and 17 may give rise to unspecific amplification products.

⁸Primer mix 7: Specific PCR fragment of 120 bp in the B*51:05, 51:08-51:09:02, 51:13:02-51:14, 51:19, 51:20, 51:31, 51:40, 51:44N, 51:54, 51:73, 51:91, 51:93, 52:03, 52:10, 52:19 and 78:04 alleles. Specific PCR fragment of 210 bp in the B*56:25 and in the B*07:78^w, 07:84^w, 13:16, 15:16, 15:42, 15:67, 15:95, 49:01:01, 49:02-49:10, 50:01:01-50:02, 50:04-50:09, 51:01:04, 51:01:13, 51:02:04 51:13:01, 51:24:01-51:24:02, 51:38, 51:92, 52:01:05, 52:14, 54:20w, 55:01:01-55:01:03, 55:01:05-55:01:06, 55:03, 55:05, 55:09w, 55:11, 55:15, 55:17, 55:24-55:25, 55:28-55:29, 55:31, 55:33, 55:36, 55:38 and 55:40 alleles. Specific PCR fragment of 120 and 210 bp in the B*56:05:01-56:06 and 56:21 and in the B*40:95, 51:01:01-51:01:03, 51:01:05-51:01:12, 51:01:14-51:02:03, 51:03, 51:07:01-51:07:02, 51:11N-51:12, 51:16-51:18, 51:21-51:23, 51:24:03, 51:26-51:30, 51:32-51:37, 51:39, 51:41N, 51:43, 51:48-51:53, 51:55, 51:57-51:58, 51:60-51:61, 51:63, 51:65-51:72, 51:74-51:80, 51:82-51:90, 51:94-51:96, 52:01:01-52:01:04, 52:02, 52:04-52:09, 52:11-52:13, 52:15-52:18, 52:20-52:21, 55:01:04, 58:08, 78:01-78:03 and 78:05-78:07 alleles.

⁹Primer mix 15: Specific PCR fragment of 160 bp in the B*56:13 and in the B*44:15, 44:18, 45:01, 45:04-45:08, 45:10-45:12 and 82:01-82:03 alleles. Specific PCR fragment of 380 bp in the B* 56:28N allele.

¹⁰Primer mix 19: Specific PCR fragment of 75 bp in the B* 56:29 allele. Specific PCR fragment of 185 bp in the B*56:19N and in the B*14:07N and 39:40N alleles.

¹¹Primer mix 21: Specific PCR fragment of 110 bp in the B* *56:17 and 56:23 and in the B*07:37 alleles. Specific PCR fragment of 170 bp in the B*56:26 allele.

¹²Primer mix 23: Specific PCR fragment of 85 bp in the B*56:20 and in the B*07:78 and 40:48 alleles. Specific PCR fragment of 350 bp in the B*56:24 allele.

'w', might be weakly amplified.

INTERPRETATION TABLE

HLA-B*56 SSP subtyping

Amplification patterns of the B*56:01 to B*56:29 alleles

	Well ⁴											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	125	400	180	105	285	105	120	395	145	150	105	180
PCR product							210					
Length of int.	800	1070	1070	800	1070	1070	800	1070	1070	800	800	800
pos. control ¹												
5'-primer ²	379	41	420	355	48	141	357	41	206	355	357	420
	5'-ACC ^{3'}	5'-CTg ^{3'}	5'-TTA ^{3'}	5'-CCC ^{3'}	5'-gCC ^{3'}	5'-ATT ^{3'}	5'-Tgg ^{3'}	5'-CTg ^{3'}	5'-AgA ^{3'}	5'-TCA ^{3'}	5'-Tgg ^{3'}	5'-TTA ^{3'}
3'-primer(s) ³	463	272	559	420	165	204	435	269	309	463	420	559
	5'-gCT ^{3'}	5'-TgT ^{3'}	5'-CAG ^{3'}	5'-gCT ^{3'}	5'-Tgg ^{3'}	5'-TCT ^{3'}	5'-TCT ^{3'}	5'-ACT ^{3'}	5'-gTg ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	5'-CgT ^{3'}
	463	277	564				527					
	5'-gCT ^{3'}	5'-ggC ^{3'}	5'-ACT ^{3'}				5'-CCT ^{3'}					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
HLA-B allele												
*56:01:01-56:01:04	1	2	3								11	
*56:02	1	2	3	4								
*56:03		2			5							
*56:04		2	3	4								
*56:05:01		2				6	7					
*56:05:02, 56:06 ⁵		2					7					
*56:07	1	2	3						9		11	
*56:08	1	2	3					8			11	
*56:09, 35:76, 35:78 ⁶	1	2								10		
*56:10	1	2		4								12
*56:11	1	2								10		
*56:12		2								10		
*56:13, 56:28N ⁷	1	2	3								11	
*56:14	1		3					8			11	
*56:15	1	2										
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE												
HLA-B*56 SSP subtyping												
Amplification patterns of the B*56:01 to B*56:29 alleles												
Well ⁴												
13	14	15	16	17	18	19	20	21	22	23	24	
180	80	160	160	180	295	75	215	110	180	85	380	Length of spec.
		380				185		170		350		PCR product
1070	1070	1070	1070	800	800	800	1070	1070	1070	1070	1070	Length of int.
												pos. control ¹
420	379	41	418	419	15	206	1 st I	206	419	420	317	5'-primer ²
5' -TTC ^{3'}	5' -ACg ^{3'}	5' -CTg ^{3'}	5' -Agg ^{3'}	5' -gTA ^{3'}	5' -gCA ^{3'}	5' -AgA ^{3'}	5' -Cag ^{3'}	5' -AgA ^{3'}	5' -gTC ^{3'}	5' -TTA ^{3'}	5' -gCT ^{3'}	
420		420				419				701		
5' -TTC ^{3'}		5' -TTA ^{3'}				5' -gTT ^{3'}				5' -CCT ^{3'}		
559	420	252	538	559	142	239	175	277	559	463	412	3'-primer(s) ³
5' -Cag ^{3'}	5' -gCT ^{3'}	5' -TCT ^{3'}	5' -gTC ^{3'}	5' -Cag ^{3'}	5' -TgA ^{3'}	5' -gCT ^{3'}	5' -CCg ^{3'}	5' -ggC ^{3'}	5' -CgT ^{3'}	5' -gCg ^{3'}	5' -gTT ^{3'}	
		538				564		335		916		
		5' -gTC ^{3'}				5' -ACT ^{3'}		5' -CgT ^{3'}		5' -gAT ^{3'}		
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												HLA-B allele
												*56:01:01-56:01:04
												*56:02
												*56:03
	14											*56:04
				17								*56:05:01
				17								*56:05:02, 56:06 ⁵
												*56:07
												*56:08
												*56:09, 35:76, 35:78 ⁶
13												*56:10
												*56:11
												*56:12
		15										*56:13, 56:28N ⁷
												*56:14
				17								*56:15
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

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Length of spec.	125	400	180	105	285	105	120	395	145	150	105	180
PCR product							210					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*56:16	1	2	3								11	
*56:17, 56:26 ⁸	1	2	3								11	
*56:18		2										
*56:19N, 56:29 ⁹	1	2	3								11	
*56:20		2	3								11	
*56:21		2					7					
*56:22	1	2										
*56:23	1	2									11	12
*56:24	1	2	3								11	
*56:25	1	2	3				7				11	
*56:27		2	3								11	
*07:02:19, 15:01:04, 40:94												
*07:20, 07:24, 07:60, 07:100, 08:21, 08:25, 15:09-15:10:02, 15:30, 15:37, 15:45, 15:48, 15:63, 15:90, 15:99, 15:150, 35:09:01-35:09:02, 35:18, 35:22, 35:44, 40:05, 40:26, 40:63, 40:92, C*03:81												
*07:37												
*07:65, 55:04, 55:20, 81:01		2										
*07:78							w				11	
*07:84							w					
*08:02, 15:115, 27:01, 38:02:01-38:04, 38:08, 38:15, 38:18, 38:23									9			
*08:03, 08:52, 40:13, 40:19, 40:109, 40:117, 48:18, 51:10, 51:81, 58:27, 59:02												
*08:37, 15:50, 15:69, 15:93, 15:186, 15:199, 18:01:01-18:09, 18:11-18:15, 18:17N-18:19, 18:23N-18:25, 18:27- 18:34, 18:37-18:40, 18:42-18:49, 39:07, 39:43, 42:09, 46:03, 46:21, C*07:123, C*15:09, C*15:19-15:20, C*15:27, C*16:20												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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180	80	160	160	180	295	75	215	110	180	85	380	Length of spec.
		380				185		170		350		PCR product
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
					18							*56:16
								21				*56:17, 56:26 ⁸
									22			*56:18
						19						*56:19N, 56:29 ⁹
										23		*56:20
				17							24	*56:21
13												*56:22
								21				*56:23
										23		*56:24
												*56:25
	14											*56:27
							20					*07:02:19, 15:01:04, 40:94
				17								*07:20, 07:24, 07:60, 07:100, 08:21, 08:25, 15:09-15:10:02, 15:30, 15:37, 15:45, 15:48, 15:63, 15:90, 15:99, 15:150, 35:09:01-35:09:02, 35:18, 35:22, 35:44, 40:05, 40:26, 40:63, 40:92, C*03:81
								21				*07:37
												*07:65, 55:04, 55:20, 81:01
										23		*07:78
												*07:84
												*08:02, 15:115, 27:01, 38:02:01-38:04, 38:08, 38:15, 38:18, 38:23
											24	*08:03, 08:52, 40:13, 40:19, 40:109, 40:117, 48:18, 51:10, 51:81, 58:27, 59:02
									22			*08:37, 15:50, 15:69, 15:93, 15:186, 15:199, 18:01:01-18:09, 18:11-18:15, 18:17N-18:19, 18:23N-18:25, 18:27-18:34, 18:37-18:40, 18:42-18:49, 39:07, 39:43, 42:09, 46:03, 46:21, C*07:123, C*15:09, C*15:19-15:20, C*15:27, C*16:20
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

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Length of spec.	125	400	180	105	285	105	120	395	145	150	105	180
PCR product							210					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*08:49, 14:10, 15:02:01-15:02:04, 15:13, 15:21, 15:25:01-15:25:02, 15:36, 15:44, 15:77, 15:80, 15:88-15:89, 15:106, 15:112, 15:121, 15:139, 15:144, 15:154, 15:165, 15:170, 27:19, 27:30, 35:16-35:17, 35:61, 35:113, 35:125, 37:01:01-37:01:03, 37:01:05-37:01:07, 37:03N-37:06, 37:08, 37:10-37:11, 37:13-37:23, 38:20, 39:42, 44:03:01-44:05:02, 44:07, 44:13-44:14, 44:17, 44:25-44:26, 44:28:01-44:30, 44:32, 44:35-44:39, 44:42-44:43, 44:45-44:46, 44:50-44:51, 44:56N-44:58N, 44:60-44:61N, 44:64:01-44:64:02, 44:69-44:70, 44:75-44:76, 44:78-44:79, 44:81, 44:85, 44:88, 44:92, 44:94, 44:96, 44:98, 44:103, 44:105, 44:108N-44:110, 51:42, 53:07, 57:01:01-57:01:04, 57:01:06-57:01:08, 57:04, 57:06, 57:08, 57:10, 57:14-57:16, 57:18-57:20, 57:22-57:25, 57:27, 57:29-57:30, 57:32, 58:10N										10		
*13:01:01-13:01:05, 13:07N, 13:12-13:13, 13:17, 13:20, 13:22:01-13:23, 13:25-13:26, 13:28-13:29, 13:36, 13:39, 15:20, 15:62, 15:85, 15:194, 35:01:01:01-35:01:21, 35:07-35:08:04, 35:10-35:11:02, 35:14:01-35:15, 35:19-35:21, 35:23-35:30, 35:33, 35:40N-35:42:02, 35:45-35:50, 35:52, 35:54, 35:57, 35:62-35:63, 35:65Q, 35:69, 35:71, 35:77, 35:80, 35:82, 35:90-35:92, 35:94, 35:96, 35:100-35:101:02, 35:103-35:105, 35:107-35:108:02, 35:110-35:112, 35:115-35:116, 35:120-35:124, 35:126, 35:130N-35:134N, 35:137-35:145N, 48:02:01-48:02:02, 53:01:01-53:03, 53:05-53:06, 53:08:01-53:13, 53:15-53:18, 53:20-53:23, 58:01:01-58:01:02, 58:01:04-58:01:07, 58:04, 58:09, 58:11-58:15, 58:17N, 58:19, 58:21-58:24, 58:29-58:30	1									10		
*13:02:01-13:02:09, 13:08Q-13:09, 13:14-13:15, 13:18-13:19, 13:27, 13:30-13:34, 13:37-13:38, 45:03	1											11
*13:03, 46:11, 49:01:02, 51:15, 59:04	1		3									11
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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180	80	160	160	180	295	75	215	110	180	85	380	Length of spec.
		380				185		170		350		PCR product
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												*08:49, 14:10, 15:02:01-15:02:04, 15:13, 15:21, 15:25:01-15:25:02, 15:36, 15:44, 15:77, 15:80, 15:88-15:89, 15:106, 15:112, 15:121, 15:139, 15:144, 15:154, 15:165, 15:170, 27:19, 27:30, 35:16-35:17, 35:61, 35:113, 35:125, 37:01:01-37:01:03, 37:01:05-37:01:07, 37:03N-37:06, 37:08, 37:10-37:11, 37:13-37:23, 38:20, 39:42, 44:03:01-44:05:02, 44:07, 44:13-44:14, 44:17, 44:25-44:26, 44:28:01-44:30, 44:32, 44:35-44:39, 44:42-44:43, 44:45-44:46, 44:50-44:51, 44:56N-44:58N, 44:60-44:61N, 44:64:01-44:64:02, 44:69-44:70, 44:75-44:76, 44:78-44:79, 44:81, 44:85, 44:88, 44:92, 44:94, 44:96, 44:98, 44:103, 44:105, 44:108N-44:110, 51:42, 53:07, 57:01:01-57:01:04, 57:01:06-57:01:08, 57:04, 57:06, 57:08, 57:10, 57:14-57:16, 57:18-57:20, 57:22-57:25, 57:27, 57:29-57:30, 57:32, 58:10N
												*13:01:01-13:01:05, 13:07N, 13:12-13:13, 13:17, 13:20, 13:22:01-13:23, 13:25-13:26, 13:28-13:29, 13:36, 13:39, 15:20, 15:62, 15:85, 15:194, 35:01:01:01-35:01:21, 35:07-35:08:04, 35:10-35:11:02, 35:14:01-35:15, 35:19-35:21, 35:23-35:30, 35:33, 35:40N-35:42:02, 35:45-35:50, 35:52, 35:54, 35:57, 35:62-35:63, 35:65Q, 35:69, 35:71, 35:77, 35:80, 35:82, 35:90-35:92, 35:94, 35:96, 35:100-35:101:02, 35:103-35:105, 35:107-35:108:02, 35:110-35:112, 35:115-35:116, 35:120-35:124, 35:126, 35:130N-35:134N, 35:137-35:145N, 48:02:01-48:02:02, 53:01:01-53:03, 53:05-53:06, 53:08:01-53:13, 53:15-53:18, 53:20-53:23, 58:01:01-58:01:02, 58:01:04-58:01:07, 58:04, 58:09, 58:11-58:15, 58:17N, 58:19, 58:21-58:24, 58:29-58:30
												*13:02:01-13:02:09, 13:08Q-13:09, 13:14-13:15, 13:18-13:19, 13:27, 13:30-13:34, 13:37-13:38, 45:03
												*13:03, 46:11, 49:01:02, 51:15, 59:04
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

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Length of spec.	125	400	180	105	285	105	120	395	145	150	105	180
PCR product							210					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*13:04, 15:34, 15:137, 27:14, 35:05:01-35:05:02, 35:37, 35:58, 35:66, 35:68:01-35:68:02, 35:72, 35:89, 35:97, 35:119, 45:02, 45:09, 53:14, 58:02, 58:06-58:07, 58:16, 58:18, 58:25-58:26, 59:03, C*06:20, C*07:81	1											
*13:06	1									10		12
*13:11	1			4								
*13:16, 49:07	1						7				11	
*14:07N, 39:40N												
*15:16, 15:67, 15:95, 49:04-49:05, 50:09	1						7					
*15:18:03				4								
*15:42	1						7				11	12
*15:58, 15:133, 39:32-39:33												
*15:73, 40:71, 51:62			3	4								
*15:86	1			4								12
*18:22, 35:35, 35:93, 58:05	1									10		
*35:02:01-35:02:04, 35:04:01-35:04:03, 35:34, 35:81, 35:83, 35:95, 35:129N, 40:28	1									10		
*35:03:01-35:03:04, 35:06, 35:13, 35:36, 35:38, 35:55-35:56, 35:70, 35:74, 35:84-35:85, 35:98, 35:106, 35:109, 35:128, 35:136, 53:04	1									10		
*35:12:01-35:12:02, 35:39, 44:62, 44:77, 44:82, 44:107, 57:12, 57:28N										10		
*35:59										10		
*35:60, 46:18, 55:18, 55:34, 59:01, 59:05	1										11	12
*35:75, 35:127	1											
*37:01:04										10		
*39:17				4								w
*40:48				4								
*40:95, 51:01:15, 51:14, 51:37, 51:63, 51:85, 78:07							7					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **35K**

Lot-specific Information

www.olerup-ssp.com

180	80	160	160	180	295	75	215	110	180	85	380	Length of spec.
		380				185		170		350		PCR product
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												*13:04, 15:34, 15:137, 27:14, 35:05:01-35:05:02, 35:37, 35:58, 35:66, 35:68:01-35:68:02, 35:72, 35:89, 35:97, 35:119, 45:02, 45:09, 53:14, 58:02, 58:06-58:07, 58:16, 58:18, 58:25-58:26, 59:03, C*06:20, C*07:81
												*13:06
												*13:11
												*13:16, 49:07
						19						*14:07N, 39:40N
												*15:16, 15:67, 15:95, 49:04-49:05, 50:09
	14											*15:18:03
												*15:42
13												*15:58, 15:133, 39:32-39:33
	14											*15:73, 40:71, 51:62
												*15:86
								22				*18:22, 35:35, 35:93, 58:05
				17								*35:02:01-35:02:04, 35:04:01-35:04:03, 35:34, 35:81, 35:83, 35:95, 35:129N, 40:28
13												*35:03:01-35:03:04, 35:06, 35:13, 35:36, 35:38, 35:55-35:56, 35:70, 35:74, 35:84-35:85, 35:98, 35:106, 35:109, 35:128, 35:136, 53:04
				17								*35:12:01-35:12:02, 35:39, 44:62, 44:77, 44:82, 44:107, 57:12, 57:28N
13												*35:59
												*35:60, 46:18, 55:18, 55:34, 59:01, 59:05
13												*35:75, 35:127
							20					*37:01:04
	14											*39:17
	14									23		*40:48
												*40:95, 51:01:15, 51:14, 51:37, 51:63, 51:85, 78:07
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

Lot No.: **35K**

Lot-specific Information

www.olerup-ssp.com

Length of spec.	125	400	180	105	285	105	120	395	145	150	105	180
PCR product							210					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*44:02:01:01-44:02:13, 44:06, 44:08-44:09, 44:11-44:12, 44:16, 44:19N, 44:21-44:24, 44:27, 44:33-44:34, 44:49, 44:52N, 44:55, 44:59, 44:63, 44:66-44:68, 44:71-44:74, 44:80, 44:83-44:84, 44:86-44:87, 44:89, 44:91, 44:93, 44:95, 44:97, 44:101-44:102, 44:104										10		
*44:10	1		3							10		
*44:15, 44:18, 45:01, 45:04-45:07, 45:11-45:12	1		3								11	
*44:20, 44:41, 44:53, 44:99-44:100, 44:106												
*44:40									9	10		
*44:44									9	10		
*44:48	1									10		
*44:90, 83:01		2								10		
*45:08	1										11	w
*45:10	1										11	
*49:01:01, 49:02-49:03, 49:06, 49:08-49:10, 50:01:01, 50:02, 50:05-50:08	1		3					7			11	
*50:01:02			3					7			11	
*50:04			3					7			11	
*51:01:01-51:01:07, 51:01:09-51:01:14, 51:01:16-51:03, 51:05, 51:07:01-51:09:02, 51:11N-51:12, 51:18-51:20, 51:22-51:24:03, 51:26-51:30, 51:32-51:33, 51:35, 51:38-51:41N, 51:43-51:44N, 51:48-51:49, 51:51-51:53, 51:55, 51:57-51:58, 51:60-51:61, 51:65-51:77, 51:79-51:80, 51:83-51:84, 51:86-51:89, 51:91, 51:94-51:96, 52:01:01-52:07, 52:09-52:11, 52:13, 52:15-52:19, 52:21, 58:08								7				
*51:01:08, 51:50, 51:54, 51:78, 51:90, 52:12, 52:20, 78:01-78:06								7				
*51:04, 51:46, 57:02:01-57:03:02, 57:07, 57:09, 57:17										10		
*51:06, 51:59, 51:64												
*51:13:01-51:13:02, 51:92, 52:14								7				
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **35K**

Lot-specific Information

www.olerup-ssp.com

180	80	160	160	180	295	75	215	110	180	85	380	Length of spec.
		380				185		170		350		PCR product
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
			16									*44:02:01:01-44:02:13, 44:06, 44:08-44:09, 44:11-44:12, 44:16, 44:19N, 44:21-44:24, 44:27, 44:33-44:34, 44:49, 44:52N, 44:55, 44:59, 44:63, 44:66-44:68, 44:71-44:74, 44:80, 44:83-44:84, 44:86-44:87, 44:89, 44:91, 44:93, 44:95, 44:97, 44:101-44:102, 44:104
												*44:10
		15										*44:15, 44:18, 45:01, 45:04-45:07, 45:11-45:12
			16									*44:20, 44:41, 44:53, 44:99-44:100, 44:106
												*44:40
			16									*44:44
			16									*44:48
			16									*44:90, 83:01
		15										*45:08
		15										*45:10
												*49:01:01, 49:02-49:03, 49:06, 49:08-49:10, 50:01:01, 50:02, 50:05-50:08
												*50:01:02
	14											*50:04
				17							24	*51:01:01-51:01:07, 51:01:09-51:01:14, 51:01:16-51:03, 51:05, 51:07:01-51:09:02, 51:11N-51:12, 51:18-51:20, 51:22-51:24:03, 51:26-51:30, 51:32-51:33, 51:35, 51:38-51:41N, 51:43-51:44N, 51:48-51:49, 51:51-51:53, 51:55, 51:57-51:58, 51:60-51:61, 51:65-51:77, 51:79-51:80, 51:83-51:84, 51:86-51:89, 51:91, 51:94-51:96, 52:01:01-52:07, 52:09-52:11, 52:13, 52:15-52:19, 52:21, 58:08
				17								*51:01:08, 51:50, 51:54, 51:78, 51:90, 52:12, 52:20, 78:01-78:06
				17							24	*51:04, 51:46, 57:02:01-57:03:02, 57:07, 57:09, 57:17
				17							24	*51:06, 51:59, 51:64
13												*51:13:01-51:13:02, 51:92, 52:14
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

Lot No.: **35K**

Lot-specific Information

www.olerup-ssp.com

Length of spec.	125	400	180	105	285	105	120	395	145	150	105	180
PCR product							210					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*51:16-51:17, 51:21, 51:31, 51:34, 51:36, 51:82, 51:93, 52:08							7					
*51:56, 53:19, 57:05, 58:28	1									10		
*54:01-54:02, 54:05N, 54:08N, 54:10, 54:12-54:13, 54:17-54:19, 54:22-54:23	1	2									11	12
*54:03	1	2	3								11	
*54:04, 54:15	1	2										
*54:06, 54:11		2										
*54:07	1										11	12
*54:09		2										
*54:14	1	2										
*54:16	1	2									11	
*54:20	1	2					w				11	12
*54:21	1	2									11	12
*55:01:01-55:01:03, 55:01:05, 55:03, 55:05, 55:11, 55:15, 55:25, 55:29, 55:31, 55:33, 55:36, 55:40	1	2					7				11	12
*55:01:04	1	2					7					12
*55:01:06		2					7				11	12
*55:02:01-55:02:03, 55:02:05-55:02:06, 55:07, 55:12, 55:16, 55:19, 55:21, 55:26, 55:30, 55:37, 55:41, 55:43	1	2									11	12
*55:02:04		2									11	12
*55:08		2										
*55:09	1	2					w				11	
*55:10	1	2									11	12
*55:13, 55:27, 55:32	1	2										
*55:14		2								10		
*55:17		2					7					
*55:22	1	2									11	
*55:23	1	2										
*55:24	1	2					7				11	
*55:28	1	2					7					
*55:35	1	2			5						11	12
*55:38		2					7				11	12
*55:39, 55:42	1	2										12
*57:26										10		
*81:03-81:04N		?										
*82:01-82:03	1	2	3	4								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **35K**

Lot-specific Information

www.olerup-ssp.com

180	80	160	160	180	295	75	215	110	180	85	380	Length of spec.
		380				185		170		350		PCR product
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
											24	*51:16-51:17, 51:21, 51:31, 51:34, 51:36, 51:82, 51:93, 52:08
				17							24	*51:56, 53:19, 57:05, 58:28
							20					*54:01-54:02, 54:05N, 54:08N, 54:10, 54:12-54:13, 54:17-54:19, 54:22-54:23
							20					*54:03
							20					*54:04, 54:15
							20					*54:06, 54:11
							20					*54:07
							20	22				*54:09
							20	22				*54:14
							20					*54:16
							20					*54:20
				18			20					*54:21
												*55:01:01-55:01:03, 55:01:05, 55:03, 55:05, 55:11, 55:15, 55:25, 55:29, 55:31, 55:33, 55:36, 55:40
												*55:01:04
												*55:01:06
												*55:02:01-55:02:03, 55:02:05-55:02:06, 55:07, 55:12, 55:16, 55:19, 55:21, 55:26, 55:30, 55:37, 55:41, 55:43
												*55:02:04
				17								*55:08
					18							*55:09
												*55:10
									22			*55:13, 55:27, 55:32
									22			*55:14
												*55:17
												*55:22
									22			*55:23
												*55:24
												*55:28
												*55:35
	14											*55:38
												*55:39, 55:42
									22			*57:26
												*81:03-81:04N
		15			18							*82:01-82:03
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

Lot No.: **35K**

Lot-specific Information

www.olerup-ssp.com

Length of spec.	125	400	180	105	285	105	120	395	145	150	105	180
PCR product							210					
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
C*02:06, C*12:15				4								
C*03:12, C*03:19, C*15:26												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

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 1 070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*56 subtyping.

In addition, wells number 4, 7, 10 to 12 and 17 to 19 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 1st, 2nd, 3rd or 4th exon or in the 1st intron, matching the specificity-determining 3'-en 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, 3rd or 5th 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.

Lot No.: **35K**

Lot-specific Information

www.olerup-ssp.com

180	80	160	160	180	295	75	215	110	180	85	380	Length of spec. PCR product
		380				185		170		350		
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												C*02:06, C*12:15
	w											C*03:12, C*03:19, C*15:26
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

⁴Primer mix 7: Specific PCR fragment of 120 bp in the B*51:05, 51:08-51:09:02, 51:13:02-51:14, 51.19, 51:20, 51:31, 51:40, 51:44N, 51:54, 51:73, 51:91, 51:93, 52:03, 52:10, 52:19 and 78:04 alleles. Specific PCR fragment of 210 bp in the B*56:25 and in the B*07:78^w, 07:84^w, 13:16, 15:16, 15:42, 15:67, 15:95, 49:01:01, 49:02-49:10, 50:01:01-50:02, 50:04-50:09, 51:01:04, 51:01:13, 51:02:04 51:13:01, 51:24:01-51:24:02, 51:38, 51:92, 52:01:05, 52:14, 54:20w, 55:01:01-55:01:03, 55:01:05-55:01:06, 55:03, 55:05, 55:09w, 55:11, 55:15, 55:17, 55:24-55:25, 55:28-55:29, 55:31, 55:33, 55:36, 55:38 and 55:40 alleles. Specific PCR fragment of 120 and 210 bp in the B*56:05:01-56:06 and 56:21 and in the B*40:95, 51:01:01-51:01:03, 51:01:05-51:01:12, 51:01:14-51:02:03, 51:03, 51:07:01-51:07:02, 51:11N-51:12, 51:16-51:18, 51:21-51:23, 51:24:03, 51:26-51:30, 51:32-51:37, 51:39, 51:41N, 51:43, 51:48-51:53, 51:55, 51:57-51:58, 51:60-51:61, 51:63, 51:65-51:72, 51:74-51:80, 51:82-51:90, 51:94-51:96, 52:01:01-52:01:04, 52:02, 52:04-52:09, 52:11-52:13, 52:15-52:18, 52:20-52:21, 55:01:04, 58:08, 78:01-78:03 and 78:05-78:07 alleles.

Primer mix 15: Specific PCR fragment of 160 bp in the B*56:13 and in the B*44:15, 44:18, 45:01, 45:04-45:08, 45:10-45:12 and 82:01-82:03 alleles. Specific PCR fragment of 380 bp in the B* 56:28N allele.

Primer mix 19: Specific PCR fragment of 75 bp in the B* 56:29 allele. Specific PCR fragment of 185 bp in the B*56:19N and in the B*14:07N and 39:40N alleles.

Primer mix 21: Specific PCR fragment of 110 bp in the B* *56:17 and 56:23 and in the B*07:37 alleles. Specific PCR fragment of 170 bp in the B*56:26 allele.

Primer mix 23: Specific PCR fragment of 85 bp in the B*56:20 and in the B*07:78 and 40:48 alleles. Specific PCR fragment of 350 bp in the B*56:24 allele.

⁵The HLA-B*56 primer set cannot separate the B*56:05:02 and B*56:06 alleles. These two alleles can be distinguished by the HLA-B low resolution kit.

⁶The HLA-B*56 primer set cannot separate the B*56:09, B*35:76 and B*35:78 alleles. These alleles can be distinguished by the HLA-B low resolution and/or B*35 kits.

⁷The B*56:13 and 56:28N alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 15.

⁸The B*56:17 and 56:26 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 21.

⁹The B*56:19N and 56:29 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 19.

'w', may be weakly amplified.

Lot No.: **35K**

Lot-specific Information

www.olerup-ssp.com

CELL LINE VALIDATION SHEET																			
HLA-B*56 SSP subtyping kit																			
			Prod. No.:	Well															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				201075001	201075002	201075003	201075004	201075005	201075006	201075007	201075008	201075009	201075010	201075011	201075012	201075013	201075014	201075015	201075016
	IHWC 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	9025 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	W	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
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	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-

Lot No.: **35K**

Lot-specific Information

CELL LINE VALIDATION SHEET				Well								
				17	18	19	20	21	22	23	24	
HLA-B*56 SSP subtyping kit												
				Prod. No.:	201075017	201075018	201075019	201075020	201075021	201075022	201075023	201075024
	IHWC 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	9025 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	-	-	-	-	-	-	-	-

Lot No.: **35K**

Lot-specific Information

www.olerup-ssp.com

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-B*56 SSP

Product number: 101.571-06 – including *Taq* polymerase
Lot number: 35K
Expiry date: 2012-September-01
Number of tests: 6
Number of wells per test: 24

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2010-750-01	9	2010-750-09	17	2010-750-17
2	2010-750-02	10	2010-750-10	18	2010-750-18
3	2010-750-03	11	2010-750-11	19	2010-750-19
4	2010-750-04	12	2010-750-12	20	2010-750-20
5	2010-750-05	13	2010-750-13	21	2010-750-21
6	2010-750-06	14	2010-750-14	22	2010-750-22
7	2010-750-07	15	2010-750-15	23	2010-750-23
8	2010-750-08	16	2010-750-16	24	2010-750-24

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

No DNAs carrying the alleles to be amplified by primer solutions 6, 8, 19, 21 and 23 were available. The specificities of the primers in primer solutions 6, 8 and 23 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 19 and 21, it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 1, 2, 3 and 15 one or two 3'-primers were not possible to test, and in primer solution 23, one 5'-primer was not possible to test. One additional 5'-primer in primer solution 15 was tested by separately adding one 3'-primer

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

Date of approval: 2011-November-28

Approved by:

Production Quality Control

Lot No.: **35K**

Lot-specific Information

www.olerup-ssp.com

Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*56
Product number: 101.571-06
Lot number: 35K

Intended use: HLA-B*56 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-November-28

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

Lot No.: **35K**

Lot-specific Information

www.olerup-ssp.com

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