

Olerup SSP® HLA-C*06

Product number:	101.614-12 – including <i>Taq</i> polymerase
Lot number:	31M
Expiry date:	2014-January-01
Number of tests:	12
Number of wells per test:	31
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. 31M.

**CHANGES COMPARED TO THE PREVIOUS OLERUP SSP®
HLA-C*06 LOT**

The HLA-C*06 specificity and interpretation tables have been updated for the HLA-C alleles described since the previous *Olerup SSP® HLA-C*06* lot was made (**Lot No. 08K**).

Seven wells have been added to the HLA-C*06 kit,
wells 25 to 31.

The amplification patterns for some rare HLA-C*06 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
5	-	-	Exchanged positive control primer pair.
25	New	New	Primer pair for the C*06:38 allele.
26	New	New	Primer pair for the C*06:39 allele.
27	New	New	Primer pairs for the C*06:40 and C*06:42 alleles.
28	New	New	Primer pair for the C*06:41 alleles.
29	New	New	Primer pair for the C*06:43 alleles.
30	New	New	Primer pair for the C*06:44 allele.
31	New	New	Primer pair for the C*06:45 allele.

PRODUCT DESCRIPTION

HLA-C*06 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-C*06:02 to HLA-C*06:45 alleles.

PLATE LAYOUT

Each test consists of 31 PCR reactions in a 32 well 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	31	empty

The 32 well cut PCR plate is marked with 'HLA-C*06' in silver/gray ink.

Well No. 1 is marked with the Lot No. '31M'.

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 32 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-C*06 SSP subtypings will be influenced by the C*01, seven C*02, the C*03, most C*04, the C*05, several C*07, the C*08, most C*12, the C*14, the C*15, two C*16, the C*17 and the C*18 alleles when present on the other haplotype. In addition, the B*58:02 allele will be amplified by primer mix 4, the B*15:137 allele will be amplified by the primer mix 9, the B*15:193 allele will be amplified by the primer mix 10 and 11, the B*08:56, B*15:142 and B*51:68 alleles will be amplified by primer mix 19 and the B*13:31, B*15:58, B*15:73, B*15:137, B*39:36 and B*55:21 alleles will be amplified by primer mix 24.

UNIQUELY IDENTIFIED ALLELES

All the HLA-C*06 alleles, i.e. C*06:02 to C*06:45, recognized by the HLA Nomenclature Committee in July 2010¹ will give rise to unique amplification patterns by the primers in the HLA-C*06 subtyping kit.

The HLA-C*06 SSP subtyping kit cannot distinguish the C*06:02:01:01, 06:02:01:02 and the C*06:02:03 to 06:02:05 alleles.

The C*06:07 and C*06:33 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 7.

The C*06:16N and C*06:21 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 16.

The C*06:24 and C*06:37 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 17.



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The C*06:25 and C*06:36 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 21.

The C*06:27 and C*06:29 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 20.

The C*06:28 and C*06:32 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 19.

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

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 73 alleles generate 38 amplification patterns that can be combined in 741 homozygous and heterozygous combinations. 208 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.

```

+++++----- *06:03, *06:05 = *06:05, *06:18 = *06:06, *06:18
+++++--- +----- *06:03, *06:23 = *06:17, *06:18 = *06:18, *06:23
+++++--- -----+ *06:18, *06:34 = *06:18, *06:35
+++++--- -----+---+ *06:18, *06:40 = *06:18, *06:42
+++++--- ----- -----+ *06:02:01:01, *06:18 = *06:03, *06:18 = *06:18,
                     *06:18
+++++--- -----+---+ *06:03, *06:34 = *06:03, *06:35
+++++--- -----+---+---+ *06:03, *06:40 = *06:03, *06:42
+++++--- +----- *06:05, *06:17 = *06:05, *06:23 = *06:06, *06:23
+++++--- +-----+ *06:09, *06:23 = *06:23, *06:34 = *06:23, *06:35
+++++--- +-----+---+ *06:23, *06:40 = *06:23, *06:42
+++++--- +----- -----+ *06:02:01:01, *06:23 = *06:17, *06:23 = *06:23,
                     *06:23
+++++--- ---+---+ ----- *06:07, *06:20 = *06:16N, *06:19 = *06:19,
                     *06:20
+++++--- ---+---+-----+ *06:19, *06:34 = *06:19, *06:35
+++++--- ---+---+---+ *06:19, *06:40 = *06:19, *06:42
+++++--- ---+----- -----+ *06:02:01:01, *06:19 = *06:07, *06:12 = *06:07,
                     *06:19 = *06:12, *06:19 = *06:19, *06:19
+++++--- -----+---+-----+ *06:07, *06:34 = *06:07, *06:35
+++++--- -----+---+---+ *06:07, *06:40 = *06:07, *06:42
+++++--- -----+-----+-----+ *06:02:01:01, *06:07 = *06:07, *06:07
+++++--- -----+---+-----+ *06:08, *06:34 = *06:08, *06:35
+++++--- -----+---+---+ *06:08, *06:40 = *06:08, *06:42
+++++--- -----+-----+-----+ *06:02:01:01, *06:08 = *06:08, *06:08
+++++--- +-----+---+-----+ *06:09, *06:40 = *06:09, *06:42
+++++--- +-----+-----+-----+ *06:02:01:01, *06:09 = *06:09, *06:09 = *06:09,
                     *06:17 = *06:09, *06:34 = *06:09, *06:35 =
                     *06:17, *06:34 = *06:17, *06:35
+++++--- +-----+---+-----+ *06:17, *06:40 = *06:17, *06:42
+++++--- +---+-----+-----+ *06:22, *06:34 = *06:22, *06:35
+++++--- +---+-----+---+-----+ *06:22, *06:40 = *06:22, *06:42
+++++--- +---+-----+-----+-----+ *06:02:01:01, *06:22 = *06:10, *06:11 = *06:10,
                     *06:22 = *06:11, *06:22 = *06:22, *06:22
+++++--- +---+-----+---+-----+ *06:10, *06:34 = *06:10, *06:35
+++++--- +---+-----+---+---+-----+ *06:10, *06:40 = *06:10, *06:42
+++++--- +---+-----+-----+-----+ *06:02:01:01, *06:10 = *06:10, *06:10
+++++--- +---+-----+---+-----+-----+ *06:11, *06:34 = *06:11, *06:35
+++++--- +---+-----+---+-----+-----+ *06:11, *06:40 = *06:11, *06:42

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+++++ ----- *06:02:01:01, *06:11 = *06:11, *06:11
 +++++ -+---+ ----- *06:20, *06:34 = *06:20, *06:35
 +++++ -+---+ ----- *06:20, *06:40 = *06:20, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:20 = *06:12, *06:16N =
 *06:12, *06:20 = *06:16N, *06:20 = *06:20,
 *06:20
 +++++ -+---+ ----- *06:12, *06:34 = *06:12, *06:35
 +++++ -+---+ ----- *06:12, *06:40 = *06:12, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:12 = *06:12, *06:12
 +++++ -+---+ ----- *06:13, *06:34 = *06:13, *06:35
 +++++ -+---+ ----- *06:13, *06:40 = *06:13, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:13 = *06:13, *06:13
 +++++ -+---+ ----- *06:15, *06:34 = *06:15, *06:35
 +++++ -+---+ ----- *06:15, *06:40 = *06:15, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:15 = *06:15, *06:15
 +++++ -+---+ ----- *06:16N, *06:34 = *06:16N, *06:35
 +++++ -+---+ ----- *06:16N, *06:40 = *06:16N, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:16N = *06:16N, *06:16N
 +++++ -+---+ ----- *06:24, *06:34 = *06:24, *06:35
 +++++ -+---+ ----- *06:24, *06:40 = *06:24, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:24 = *06:24, *06:24
 +++++ -+---+ ----- *06:26, *06:34 = *06:26, *06:35
 +++++ -+---+ ----- *06:26, *06:40 = *06:26, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:26 = *06:26, *06:26
 +++++ -+---+ ----- *06:28, *06:34 = *06:28, *06:35
 +++++ -+---+ ----- *06:28, *06:40 = *06:28, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:28 = *06:28, *06:28
 +++++ -+---+ ----- *06:27, *06:34 = *06:27, *06:35
 +++++ -+---+ ----- *06:27, *06:40 = *06:27, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:27 = *06:27, *06:27
 +++++ -+---+ ----- *06:25, *06:34 = *06:25, *06:35
 +++++ -+---+ ----- *06:25, *06:40 = *06:25, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:25 = *06:25, *06:25
 +++++ -+---+ ----- *06:30, *06:34 = *06:30, *06:35
 +++++ -+---+ ----- *06:30, *06:40 = *06:30, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:30 = *06:30, *06:30
 +++++ -+---+ ----- *06:31, *06:34 = *06:31, *06:35
 +++++ -+---+ ----- *06:31, *06:40 = *06:31, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:31 = *06:31, *06:31
 +++++ -+---+ ----- *06:34, *06:38 = *06:35, *06:38
 +++++ -+---+ ----- *06:34, *06:39 = *06:35, *06:39
 +++++ -+---+ ----- *06:34, *06:40 = *06:34, *06:42 = *06:35, *06:42
 +++++ -+---+ ----- *06:34, *06:41 = *06:35, *06:41
 +++++ -+---+ ----- *06:34, *06:43 = *06:35, *06:43
 +++++ -+---+ ----- *06:34, *06:44 = *06:35, *06:44
 +++++ -+---+ ----- *06:34, *06:45 = *06:35, *06:45
 +++++ -+---+ ----- *06:02:01:01, *06:34 = *06:02:01:01, *06:35 =
 *06:34, *06:34 = *06:34, *06:35
 +++++ -+---+ ----- *06:38, *06:40 = *06:38, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:38 = *06:38, *06:38
 +++++ -+---+ ----- *06:39, *06:40 = *06:39, *06:42
 +++++ -+---+ ----- *06:02:01:01, *06:39 = *06:39, *06:39
 +++++ -+---+ ----- *06:40, *06:41 = *06:41, *06:42
 +++++ -+---+ ----- *06:40, *06:43 = *06:42, *06:43
 +++++ -+---+ ----- *06:40, *06:44 = *06:42, *06:44
 +++++ -+---+ ----- *06:40, *06:45 = *06:42, *06:45
 +++++ -+---+ ----- *06:02:01:01, *06:40 = *06:02:01:01, *06:42 =
 *06:40, *06:42 = *06:42, *06:42



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+++++ ----- *06:02:01:01, *06:41 = *06:41, *06:41
+++++ ----- *06:02:01:01, *06:43 = *06:43, *06:43
+++++ ----- *06:02:01:01, *06:44 = *06:44, *06:44
+++++ ----- *06:02:01:01, *06:45 = *06:45, *06:45
+++-+--- ----- *06:05, *06:05 = *06:05, *06:06

*06:02:01:01 = *06:02:01:01-06:02:01:02, 06:02:03-06:02:08

*06:07 = *06:07 and 06:33

*06:16N = *06:16N and 06:21

*06:24 = *06:24 and 06:37

*06:25 = *06:25 and 06:36

*06:27 = *06:27 and 06:29

*06:28 = *06:28 and 06:32

SPECIFICITY TABLE**HLA-C*06 SSP subtyping**

Specificities and sizes of the PCR products of the 31 primer mixes used for HLA-C*06 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-C*06 alleles ³	Other amplified HLA Class I alleles ⁴
1	240 bp	800 bp	*06:02:01:01-06:02:01:02, 06:02:03-06:02:08, 06:04-06:16N, 06:18-06:45	*03:39, 03:67, 04:42, 05:43, 12:16, 16:21
2	220 bp	800 bp	*06:02:01:01-06:02:01:02, 06:02:03-06:03, 06:07-06:13, 06:15-06:34, 06:36-06:39, 06:41-06:45	*01:04, 01:09, 02:05, 02:17, 12:03-01:01-12:07, 12:11-12:13, 12:15, 12:23, 12:25-12:26, 12:28-12:29, 12:31-12:35, 12:37-12:39N, 12:42Q-12:43, 14:16, 16:04:01
3 ⁵	135 bp	1070 bp	*06:03, 06:18	*02:02:13, 03:02:01-03:04:14, 03:04:16-03:17, 03:19-03:38:02, 03:40-03:66, 03:67 ^w , 03:68-03:94, 07:96
4	250 bp	1070 bp	*06:04	*01:22, 01:35, 05:11, 05:17, 05:27, 08:01:01-08:01:02, 08:03:01-08:04, 08:06, 08:08-08:11, 08:13-08:14, 08:16, 08:20-08:22, 08:24, 08:26N, 08:36N, 12:14:01-12:14:02, 12:18, 12:20, 14:06, 14:15, 15:02:01-15:07, 15:09-15:13, 15:15-15:24, 15:26-15:35, 17:01:01:01-17:07, B*58:02
5 ⁸	165 bp, 220 bp	1070 bp	*06:05, 06:18, 06:23	*01:04, 01:09, 02:21, 05:01:01:01-05:01:13, 05:03-05:28, 05:30-05:45, 08:10, 12:21, 12:33, 17:05
6	250 bp	1070 bp	*06:05-06:06	*01:02:01-01:03, 01:05-01:08, 01:10-01:20, 01:23-01:34, 01:36-01:40, 02:32, 04:01:01:01-04:01:21, 04:03, 04:05, 04:07-04:12, 04:15:01-04:20, 04:23-04:33,

				04:35-04:47, 04:49-04:57, 04:59Q-04:67, 04:69-04:70, 05:01:01:01-05:01:13, 05:03- 05:07N, 05:09-05:10, 05:12- 05:16, 05:18-05:26, 05:28- 05:45, 08:02:01-08:02:04, 08:05, 08:07, 08:12, 08:15:01, 08:17-08:19, 08:23, 08:25, 08:28, 08:30, 08:32-08:35, 12:09, 12:24, 14:02:01-14:05, 14:07N-14:14, 14:17-14:21N, 15:08, 18:01-18:02
7 ^{5,9}	110 bp, 185 bp, 235 bp	1070 bp	*06:07, 06:19, 06:33	
8	240 bp	1070 bp	*06:08	*01:10, 02:05, 02:17
9 ¹⁰	165 bp, 210 bp, 435 bp	1070 bp	*06:09, 06:17, 06:23	*01:04, 01:09, 02:22, 05:08, 07:07, 07:09, 07:76, 08:27, 08:29, 08:31, 12:31, 18:01- 18:03, B*15:137
10 ⁶	190 bp	800 bp	*06:10, 06:22	*07:107, B*15:193
11 ^{5,11}	130 bp, 185 bp	1070 bp	*06:11, 06:22	*07:04:01-07:04:04, 07:11- 07:12, 07:45, 07:63, 07:68, 07:101, 07:107, 07:139, 07:142, 12:03:09, B*15:193
12 ^{6,12}	195 bp, 275 bp	1070 bp	*06:12, 06:19- 06:20	*01:32, 07:81
13	155 bp	1070 bp	*06:13	
14	305 bp	1070 bp	*06:14	*03:32, 03:45, 07:43, 15:25
15	360 bp	800 bp	*06:15	
16 ^{7,13}	235 bp, 275 bp, 340 bp	1070 bp	*06:16N, 06:20- 06:21	*01:32, 07:81
17 ^{7,14}	160 bp, 225 bp	1070 bp	*06:24, 06:37	*01:20
18	220 bp	1070 bp	*06:26	
19 ^{5,15}	85 bp, 160 bp	800 bp	*06:28, 06:32	*02:14, 03:67, 04:42, 05:43, 07:20, 07:73, 15:23, 16:21, B*08:56, B*15:142, B*51:68
20 ^{5,16}	115 bp, 275 bp	1070 bp	*06:27, 06:29	*07:134
21 ¹⁷	190 bp, 380 bp	1070 bp	*06:25, 06:36	
22	170 bp	1070 bp	*06:30	*02:02:13, 12:03:09
23	205 bp	1070 bp	*06:31	*16:04:01

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24¹⁸	160 bp, 210 bp	1070 bp	*06:09, 06:34- 06:35	*02:22, 05:08, 08:27, 08:29, 08:31, 12:31, 18:03, B*13:31, B*15:58, B*15:73, B*15:137, B*39:36, B*55:21
25⁵	85 bp	1070 bp	*06:38	
26⁵	130 bp	1070 bp	*06:39	
27¹⁹	190 bp, 225 bp	1070 bp	*06:40, 06:42	
28⁵	90 bp	1070 bp	*06:41	*12:32
29	175 bp	1070 bp	*06:43	
30⁵	100 bp	1070 bp	*06:44	
31	155 bp	1070 bp	*06:45	

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-C*06 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 inherit feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

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-C*06 subtyping.

In addition, wells number 2, 10, 15 and 19 contain the primer pair giving rise to the shorter, 800 bp, internal positive control in order to allow kit identification.

In the presence of a specific amplification the intensity of the control band often decreases.

³The C*06:07 and C*06:33 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 7.

The C*06:16N and C*06:21 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 16.

The C*06:24 and C*06:37 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 17.

The C*06:25 and C*06:36 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 21.

The C*06:27 and C*06:29 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 20.

The C*06:28 and C*06:32 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-C alleles non-HLA-C*06 alleles will be amplified by primer mixes 1 to 6, 8 to 12, 14, 16, 17, 19, 20, 22 to 24 and 28. In addition, the B*58:02 allele will be amplified by primer mix 4, the B*15:137 allele will be amplified by the

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primer mix 9, the B*15:193 allele will be amplified by the primer mix 10 and 11, the B*08:56, B*15:142 and B*51:68 alleles will be amplified by primer mix 19 and the B*13:31, B*15:58, B*15:73, B*15:137, B*39:36 and B*55:21 alleles will be amplified by primer mix 24.

⁵Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁶Primer mixes 10 and 12 have a tendency of giving rise to non-specific amplifications.

⁷Primer mixes 16 and 17 has a tendency of giving rise to primer oligomer formation.

⁸Primer mix 5: Specific PCR fragment of 165 bp in the C*06:05 and the C*05:01:01:01-05:01:13, 05:03-05:28, 05:30-05:45, 08:10, 12:21, 12:33 and 17:05 alleles. Specific PCR fragment of 220 bp in the C*06:18 and 06:23 and the C*01:04, 01:09 and 02:21 alleles.

⁹Primer mix 7: Specific PCR fragment of 110 bp in the C*06:07 allele. Specific PCR fragment of 185 bp in the C*06:19 allele. Specific PCR fragment of 235 bp in the C*06:33 allele.

¹⁰Primer mix 9: Specific PCR fragment of 165 bp in the C*06:09 and in the C*02:22, 05:08, 08:27, 08:29, 08:31 and 12:31 alleles, and in addition in the B*15:137 allele. Specific PCR fragment of 210 bp in the C*06:23 and the C*01:04 and 01:09 alleles. Specific PCR fragment of 435 bp in the C*06:17, 07:07, 07:09, 07:76, 18:01-18:02 alleles. Specific PCR fragments of 165 and 435 bp in the C*18:03 allele.

¹¹Primer mix 11: Specific PCR fragment of 130 bp in the C*06:11 and the C*07:04:01-07:04:04, 07:11-07:12, 07:45, 07:63, 07:68, 07:101, 07:139, 07:142 and 12:03:09 alleles. Specific PCR fragment of 185 bp in the C*06:22 and C*07:107 and in the B*15:193 allele.

¹²Primer mix 12: Specific PCR fragment of 185 bp in the C*06:19 allele. Specific PCR fragment of 205 bp in the C*06:12 allele. Specific PCR fragment of 275 bp in the C*06:20 and in the C*01:32 and 07:81 alleles.

¹³Primer mix 16: Specific PCR fragment of 235 bp in the C*06:16N allele. Specific PCR fragment of 275 bp in the C*06:20 and in the C*01:32 and 07:81 alleles. Specific PCR fragment of 340 bp in the C*06:21 allele.

¹⁴Primer mix 17: Specific PCR fragment of 160 bp in the C*06:37 and the C*01:20 alleles. Specific PCR fragment of 225 bp in the C*06:24 allele.

¹⁵Primer mix 19: Specific PCR fragment of 85 bp in the C*06:28 and the C*02:14, 03:67, 04:42, 05:43, 07:20, 07:73, 15:23 and 16:21 and in the B*08:56, B*15:142 and B*51:68 alleles. Specific PCR fragment of 160 bp in the C*06:32 allele.

¹⁶Primer mix 20: Specific PCR fragment of 115 bp in the C*06:29 and the C*07:134 alleles. Specific PCR fragment of 275 bp in the C*06:27 allele.

¹⁷Primer mix 21: Specific PCR fragment of 190 bp in the C*06:36 allele. Specific PCR fragment of 380 bp in the C*06:25 allele.

¹⁸Primer mix 24: Specific PCR fragment of 160 bp in the C*06:09 and 06:34 and the C*02:22, 05:08, 08:27, 08:29, 08:31, 12:31 and 18:03 and in the B*13:31, B*15:58, B*15:73, B*15:137, B*39:36 and B*55:21 alleles. Specific PCR fragment of 210 bp in the C*06:35 allele.

¹⁹Primer mix 27: Specific PCR fragment of 190 bp in the C*06:42 allele. Specific PCR fragment of 225 bp in the C*06:40 allele.

'w', may be weakly amplified.

INTERPRETATION TABLE**HLA-C*06 SSP subtyping**

Amplification patterns of the C*06:02 to C*06:45 alleles

				Well ¹¹															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length of spec.																			
PCR product(s)																			
Length of int.																			
pos. control ¹																			
5'-primer(s) ²																			
3'-primer(s) ³																			
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16			
HLA-C allele ⁴	1	2																	
*06:02:01:01-06:02:01:02, 06:02:03-06:02:08	1	2																	
*06:03		2	3																
*06:04	1			4	5	6													
*06:05	1				5	6													
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16			



INTERPRETATION TABLE

HLA-C*06 SSP subtyping

Amplification patterns of the C*06:02 to C*06:45 alleles



Length of spec.	240	220	135	250	220	165	250	110	240	435	210	165	190	130	275	195	155	305	360	340	275	235
PCR product(s)																						
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16						
*06:06	1					6																
*06:07, 06:33 ⁵	1	2					7															
*06:08	1	2						8														
*06:09	1	2							9													
*06:10	1	2								10												
*06:11	1	2									11											
*06:12	1	2										12										
*06:13	1	2											13									
*06:14	1													14								
*06:15	1	2													15							
*06:16N, 06:21 ⁶	1	2																				16
*06:17		2							9													
*06:18	1	2	3		5																	
*06:19	1	2				7									12							
*06:20	1	2													12			16				
*06:22	1	2							10 11													
*06:23	1	2			5				9													
*06:24, 06:37 ⁷	1	2																				
*06:25, 06:36 ⁸	1	2																				
*06:26	1	2																				
*06:27, 06:29 ⁹	1	2																				
*06:28, 06:32 ¹⁰	1	2																				
*06:30	1	2																				
*06:31	1	2																				
*06:34	1	2																				
*06:35	1																					
*06:38	1	2																				
*06:39	1	2																				
*06:40	1																					
*06:41	1	2																				
*06:42	1	2																				
*06:43	1	2																				
*06:44	1	2																				
*06:45	1	2																				
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16						

225	160	220	85	115	380	190	170	205	160	85	130	225	190	90	175	100	155			Length of spec.	PCR product(s)
17	18	19	20		21	22	23	24		25	26	27	28	29	30	31				Well No.	
																				*06:06	
																				*06:07, 06:33 ⁵	
																				*06:08	
																				*06:09	
																				*06:10	
																				*06:11	
																				*06:12	
																				*06:13	
																				*06:14	
																				*06:15	
																				*06:16N, 06:21 ⁶	
																				*06:17	
																				*06:18	
																				*06:19	
																				*06:20	
																				*06:22	
																				*06:23	
17																				*06:24, 06:37 ⁷	
					21															*06:25, 06:36 ⁸	
18																				*06:26	
			20																	*06:27, 06:29 ⁹	
		19																		*06:28, 06:32 ¹⁰	
				22																*06:30	
					23															*06:31	
						24														*06:34	
						24														*06:35	
							25													*06:38	
							26													*06:39	
								27												*06:40	
								28												*06:41	
								27												*06:42	
									29											*06:43	
										30										*06:44	
										31										*06:45	
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31						Well No.	

Length of spec.	240	220	135	250	165	250	110	240	165	210	190	130	275	195	155	305	360	235	
PCR product(s)					220	235	185			435	185							340	275
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16			
*01:02:01-01:03, 01:05-01:08, 01:11-01:19, 01:23-01:31, 01:33- 01:34, 01:36-01:40, 02:32, 04:01:01:01-04:01:21, 04:03, 04:05, 04:07-04:12, 04:15:01- 04:20, 04:23-04:33, 04:35-04:41, 04:43-04:47, 04:49-04:57, 04:59Q- 04:67, 04:69-04:70, 05:29, 08:02:01-08:02:04, 08:05, 08:07, 08:12, 08:15:01, 08:17-08:19, 08:23, 08:25, 08:28, 08:30, 08:32- 08:35, 12:09, 12:24, 14:02:01- 14:05, 14:07N-14:14, 14:17- 14:21N, 15:08																			
*01:04, 01:09	2				5					9									
*01:10						6			6		8								
*01:20							6												
*01:22, 01:35, 08:01:01-08:01:02, 08:03:01-08:04, 08:06, 08:08- 08:09, 08:11, 08:13-08:14, 08:16, 08:20-08:22, 08:24, 08:26N, 08:36N, 12:14:01-12:14:02, 12:18, 12:20, 14:06, 14:15, 15:02:01- 15:07, 15:09-15:13, 15:15-15:22, 15:24, 15:26-15:35, 17:01:01:01- 17:04, 17:06-17:07, B*58:02					4														
*01:32							6						12				16		
*02:02:13					3														
*02:05, 02:17		2						8											
*02:14, 07:20, 07:73, B*08:56, B*15:142, B*51:68																			
*02:21, 12:21						5													
*02:22, 08:27, 08:29, 08:31, 18:03, B*15:137												9							
*03:02:01-03:04:14, 03:04:16- 03:17, 03:19-03:31, 03:33- 03:38:02, 03:40-03:44, 03:46- 03:66, 03:68-03:94, 07:96			3																
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16			

Lot No.: 31M

Lot-specific information

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				Length of spec.											
				PCR product(s)											
				Well No.											
17	18	19	20	21	22	23	24	25	26	27	28				
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	*01:02:01-01:03, 01:05-01:08, 01:11-01:19, 01:23-01:31, 01:33- 01:34, 01:36-01:40, 02:32, 04:01:01:01-04:01:21, 04:03, 04:05, 04:07-04:12, 04:15:01- 04:20, 04:23-04:33, 04:35-04:41, 04:43-04:47, 04:49-04:57, 04:59Q- 04:67, 04:69-04:70, 05:29, 08:02:01-08:02:04, 08:05, 08:07, 08:12, 08:15:01, 08:17-08:19, 08:23, 08:25, 08:28, 08:30, 08:32- 08:35, 12:09, 12:24, 14:02:01- 14:05, 14:07N-14:14, 14:17- 14:21N, 15:08 *01:04, 01:09 *01:10
17														*01:20	
														*01:22, 01:35, 08:01:01-08:01:02, 08:03:01-08:04, 08:06, 08:08- 08:09, 08:11, 08:13-08:14, 08:16, 08:20-08:22, 08:24, 08:26N, 08:36N, 12:14:01-12:14:02, 12:18, 12:20, 14:06, 14:15, 15:02:01- 15:07, 15:09-15:13, 15:15-15:22, 15:24, 15:26-15:35, 17:01:01:01- 17:04, 17:06-17:07, B*58:02 *01:32	
				22										*02:02:13	
														*02:05, 02:17	
	19													*02:14, 07:20, 07:73, B*08:56, B*15:142, B*51:68 *02:21, 12:21	
					24									*02:22, 08:27, 08:29, 08:31, 18:03, B*15:137	
														*03:02:01-03:04:14, 03:04:16- 03:17, 03:19-03:31, 03:33- 03:38:02, 03:40-03:44, 03:46- 03:66, 03:68-03:94, 07:96	
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Well No.



Length of spec.	240	220	135	250	220	165	250	110	240	435	210	165	190	130	275	195	155	305	360	340	275	235
PCR product(s)																						
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16						
*03:32, 03:45			3																		14	
*03:39, 12:16		1																				
*03:67	1		w																			
*04:42	1						6															
*05:01:01:01-05:01:13, 05:03-05:07N, 05:09-05:10, 05:12-05:16, 05:18-05:26, 05:28, 05:30-05:42, 05:44-05:45						5	6															
*05:08						5					9											
*05:11, 05:17, 05:27, 08:10, 17:05				4	5																	
*05:43	1				5	6																
*07:04:01-07:04:04, 07:11-07:12, 07:45, 07:63, 07:68, 07:101, 07:139, 07:142													11									
*07:07, 07:09, 07:76											9											
*07:43, 15:25																			14			
*07:81													12						16			
*07:107, B*15:193												10	11									
*07:134																						
*12:03:01:01-12:03:08, 12:03:10-12:07, 12:11-12:13, 12:15, 12:23, 12:25-12:26, 12:28-12:29, 12:34-12:35, 12:37-12:39N, 12:42Q-12:43, 14:16			2																			
*12:03:09		2											11									
*12:31	2										9											
*12:32	2																					
*12:33	2				5																	
*15:23				4																		
*16:04:01		2																				
*16:21	1																					
*18:01-18:02								6			9											
B*13:31, B*15:58, B*15:73, B*39:36, B*55:21																						
HLA-C allele ⁴																						
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16						

																								Length of spec.	
																								PCR product(s)	
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31											Well No.
																									*03:32, 03:45
																									*03:39, 12:16
19																									*03:67
19																									*04:42
																									*05:01:01:01-05:01:13, 05:03-05:07N, 05:09-05:10, 05:12-05:16, 05:18-05:26, 05:28, 05:30-05:42, 05:44-05:45
				24																					*05:08
																									*05:11, 05:17, 05:27, 08:10, 17:05
19																									*05:43
																									*07:04:01-07:04:04, 07:11-07:12, 07:45, 07:63, 07:68, 07:101, 07:139, 07:142
																									*07:07, 07:09, 07:76
																									*07:43, 15:25
																									*07:81
																									*07:107, B*15:193
20																									*07:134
																									*12:03:01:01-12:03:08, 12:03:10-12:07, 12:11-12:13, 12:15, 12:23, 12:25-12:26, 12:28-12:29, 12:34-12:35, 12:37-12:39N, 12:42Q-12:43, 14:16
				22																					*12:03:09
					24																				*12:31
																									*12:32
																									*12:33
19																									*15:23
					23																				*16:04:01
19																									*16:21
																									*18:01-18:02
								24																	B*13:31, B*15:58, B*15:73, B*39:36, B*55:21
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31											HLA-C allele ⁴
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31											Well No.

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

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-C*06 subtyping.

In addition, wells number 2, 5, 10, 15 and 19 contain the primer pair giving rise to the shorter, 800 bp, internal positive control in order to allow kit identification.

²The nucleotide position, in the 1st, 2nd or 3rd exons or the the 2nd intron, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

³The nucleotide position, in the 2nd or 3rd exons or the 3rd intron, 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.

⁴The sequence of the C*0601 allele has been shown to be identical to C*06:02.

The sequence of the C*060202 allele has been renamed to C*06:17.

⁵The C*06:07 and C*06:33 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 7.

⁶The C*06:16N and C*06:21 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 16.

⁷The C*06:24 and C*06:37 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 17.

⁸The C*06:25 and C*06:36 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 21.

⁹The C*06:27 and C*06:29 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 20.

¹⁰The C*06:28 and C*06:32 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 19.

¹¹Primer mix 5: Specific PCR fragment of 165 bp in the C*06:05 and the C*05:01:01:01-05:01:13, 05:03-05:28, 05:30-05:45, 08:10, 12:21, 12:33 and 17:05 alleles. Specific PCR fragment of 220 bp in the C*06:18 and 06:23 and the C*01:04, 01:09 and 02:21 alleles.

Primer mix 7: Specific PCR fragment of 110 bp in the C*06:07 allele. Specific PCR fragment of 185 bp in the C*06:19 allele. Specific PCR fragment of 235 bp in the C*06:33 allele.

Primer mix 9: Specific PCR fragment of 165 bp in the C*06:09 and in the C*02:22, 05:08, 08:27, 08:29, 08:31 and 12:31 alleles, and in addition in the B*15:137 allele. Specific PCR fragment of 210 bp in the C*06:23 and the C*01:04 and 01:09 alleles. Specific PCR fragment of 435 bp in the C*06:17, 07:07, 07:09, 07:76, 18:01-18:02 alleles. Specific PCR fragments of 165 and 435 bp in the C*18:03 allele.

Primer mix 11: Specific PCR fragment of 130 bp in the C*06:11 and the C*07:04:01-07:04:04, 07:11-07:12, 07:45, 07:63, 07:68, 07:101, 07:139, 07:142 and 12:03:09 alleles. Specific PCR fragment of 185 bp in the C*06:22 and C*07:107 and in the B*15:193 allele.

Primer mix 12: Specific PCR fragment of 185 bp in the C*06:19 allele. Specific PCR fragment of 205 bp in the C*06:12 allele. Specific PCR fragment of 275 bp in the C*06:20 and in the C*01:32 and 07:81 alleles.

Primer mix 16: Specific PCR fragment of 235 bp in the C*06:16N allele. Specific PCR fragment of 275 bp in the C*06:20 and in the C*01:32 and 07:81 alleles. Specific PCR fragment of 340 bp in the C*06:21 allele.

Primer mix 17: Specific PCR fragment of 160 bp in the C*06:37 and the C*01:20 alleles. Specific PCR fragment of 225 bp in the C*06:24 allele.

Primer mix 19: Specific PCR fragment of 85 bp in the C*06:28 and the C*02:14, 03:67, 04:42, 05:43, 07:20, 07:73, 15:23 and 16:21 and in the B*08:56, B*15:142 and B*51:68 alleles. Specific PCR fragment of 160 bp in the C*06:32 allele.

Primer mix 20: Specific PCR fragment of 115 bp in the C*06:29 and the C*07:134 alleles. Specific PCR fragment of 275 bp in the C*06:27 allele.

Primer mix 21: Specific PCR fragment of 190 bp in the C*06:36 allele. Specific PCR fragment of 380 bp in the C*06:25 allele.

Primer mix 24: Specific PCR fragment of 160 bp in the C*06:09 and 06:34 and the C*02:22, 05:08, 08:27, 08:29, 08:31, 12:31 and 18:03 and in the B*13:31, B*15:58, B*15:73, B*15:137, B*39:36 and B*55:21 alleles. Specific PCR fragment of 210 bp in the C*06:35 allele.

Primer mix 27: Specific PCR fragment of 190 bp in the C*06:42 allele. Specific PCR fragment of 225 bp in the C*06:40 allele.

'w', may be weakly amplified.

CELL LINE VALIDATION SHEET

HLA-C*06 SSP subtyping kit



CELL LINE VALIDATION SHEET												
HLA-C*06 SSP subtyping kit												
			Prod. No.:	Well								
				17	18	19	20	21	22	23	24	25
1	9001	SA	*07:02	-	-	-	-	-	-	-	-	-
2	9280	LK707	*07:01	*15:05	-	-	-	-	-	-	-	-
3	9011	E4181324	*12:02	-	-	-	-	-	-	-	-	-
4	9275	GU373	*03:04	*04:01	-	-	-	-	-	-	-	-
5	9009	KAS011	*06:02	-	-	-	-	-	-	-	-	-
6	9353	SM	*03:04	*07:02	-	-	-	-	-	-	-	-
7	9020	QBL	*05:01	-	-	-	-	-	-	-	-	-
8	9025	DEU	*04:01	-	-	-	-	-	-	-	-	-
9	9026	YAR	*12:03	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*01:02	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*16:01	-	-	-	-	-	-	-	-	-
12	9052	DBB	*06:02	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*01:02	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*01:02	*03:04	-	-	-	-	-	-	-	-
15	9075	DKB	*03:04	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*02:02	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*03:03	*07:01	-	-	-	-	-	-	-	-
18	9257	32367	*01:02	*07:05	-	-	-	-	-	-	-	-
19	9038	BM16	*07:01	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*03:04	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*03:03	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*12:03	-	-	-	-	-	-	-	-	-
23	9124	IHL	*01:02	*15:02	-	-	-	-	-	-	-	-
24	9035	JBUSH	*12:03	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*08:02	-	-	-	-	-	-	-	-	-
26	9285	WT49	*07:01	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*07:04	*15:05	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*05:01	*16:01	-	-	-	-	-	-	-	-
29	9050	MOU	*16:01	-	-	-	-	-	-	-	-	-
30	9021	RSH	*17:01	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*05:01	-	-	-	-	-	-	-	-	-
32	9297	HAG	*17:01	*17:03	-	-	-	-	-	-	-	-
33	9098	MT14B	*03:04	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*12:03	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*05:01	-	-	-	-	-	-	-	-	-
36	9024	KT17	*03:03	*04:01	-	-	-	-	-	-	-	-
37	9065	HHKB	*07:02	-	-	-	-	-	-	-	-	-
38	9099	LZL	*03:03	-	-	-	-	-	-	-	-	-
39	9315	CML	*02:02	*07:01	-	-	-	-	-	-	-	-
40	9134	WHONP199	*01:02	*06:02	-	-	-	-	-	-	-	-
41	9055	H0301	*08:02	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*01:02	-	-	-	-	-	-	-	-	-
43	9076	T7526	*01:02	*08:01	-	-	-	-	-	-	-	-
44	9057	TEM	*12:03	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*06:02	*17:01	-	-	-	-	-	-	-	-
46	9013	SCHU	*07:02	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*07:04	*15:02	-	-	-	-	-	-	-	-
48	9303	TER-ND	*04:01	*16:01	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-C*06 SSP

Product number: 101.614-12 – including *Taq* polymerase
Lot number: 31M
Expiry date: 2014-January-01
Number of tests: 12
Number of wells per test: 31

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2011-872-01	13	2011-872-13	25	2011-872-25
2	2011-872-02	14	2011-872-14	26	2011-872-26
3	2011-872-03	15	2011-872-15	27	2011-872-27
4	2011-872-04	16	2011-872-16	28	2011-872-28
5	2011-872-05	17	2011-872-17	29	2011-872-29
6	2011-872-06	18	2011-872-18	30	2011-872-30
7	2011-872-07	19	2011-872-19	31	2011-872-31
8	2011-872-08	20	2011-872-20		
9	2011-872-09	21	2011-872-21		
10	2011-872-10	22	2011-872-22		
11	2011-872-11	23	2011-872-23		
12	2011-872-12	24	2011-872-24		

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 7, 8, 10, 12 to 22 and 24 to 31 were available. The specificity of the primers in primer solutions 8, 10, 12, 14, 16, 19, 21, 22, 24, 27 and 30 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 13, 17, 20 and 25 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 7, 15, 18, 26, 28, 29 and 31 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solution 3, 5, 10, 12, 16 and 21 one or two 5'-primers were not possible to test, and in primer solutions 10, 11, 19, 21 and 27 one 3'-primer was not possible to test.

Additional primers in primers solutions 5, 9 and 11 were tested by separately adding one 5'-primer or one 3'-primer.

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

Date of approval: 2011-July-15

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: Olerup SSP® HLA-C*06

Product number: 101.614-12

Lot number: 31M

Intended use: HLA-C*06 high resolution histocompatibility testing

Manufacturer: Olerup SSP AB
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We, Olerup SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2008 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex III, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at Olerup SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.

The Authorized Representative located within the Community is: Olerup SSP AB.

Stockholm, Sweden
2011-July-15

Olle Olerup

HLA-C*06

101.614-12 – including Taq polymerase

Lot No.: 31M

Product Insert

Lot-specific information

General “Instructions for Use”
IFU-01 Rev. No. 02 can be downloaded from

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www.olerup-ssp.com

July 2011
Rev. No.: 00



For *In Vitro* Diagnostic Use

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