

Olerup SSP[®] HLA-A*23

Product number:	101.421-06u – without <i>Taq</i> polymerase
Lot number:	54M
Expiry date:	2014-March-01
Number of tests:	6
Number of wells per test:	32
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. 54M.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] HLA-A*23 LOT

The HLA-A*23 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP*[®] HLA-A*23 lot was made (Lot No. 71K).

Eight wells have been added to the HLA-A*23 kit, wells **25 to 32**.
The amplification patterns for some rare HLA-A*23 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
10	-	Added	Primer added for the A*23:24 allele.
17	-	Added	Primer added for the A*23:33 allele.
22	Modified	-	Improved specificity of primer pair.
23	Added	Added	Primer pair added for the A*23:27 allele.
24	-	Added	Primer added for the A*23:40 allele.
25	New	New	New primer pairs for the A*23:41 and A*23:42 alleles.
26	New	New	New primer pairs for the A*23:36 and A*23:39 alleles.
27	New	New	New primer pairs for the A*23:34 and A*23:35 alleles.
28	New	New	New primer pair for the A*23:32 allele.

29	New	New	New primer pairs for the A*23:37 and A*23:40 alleles.
30	New	New	New primer pair for the A*23:38N allele.
31	New	New	New primer pair for the A*23:44 allele.
32	New	New	New primer pair for the A*23:46 allele.

Change in revision R01 compared to R00:

1. Primer mix 21 does not amplify the A*23:05 allele. Furthermore, for many A*23 alleles it is not known whether they are amplified by this primer mix due to lack of nucleotide sequence information. This has been changed in the specificity and amplification tables.

Change in revision R02 compared to R01:

1. A typing error on page 1 in the section specifying the number of new wells has been corrected.

Change in revision R03 compared to R02:

1. Primer mix 12 weakly amplifies the following HLA-B alleles: B*13:39, 38:16, 40:13, 40:19, 40:37, 40:109, 44:02:19, 44:05:03, 44:18, 44:25, 44:50, 44:75, 44:95, 49:01:01-49:01:03, 49:04-49:20, 51:103, 51:119 and 57:51. This has been corrected in the Specificity and Interpretation Tables.

PRODUCT DESCRIPTION

HLA-A*23 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the A*23:01 to A*23:46 alleles.

PLATE LAYOUT

Each test consists of 32 PCR reactions in a 32 well cut 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
25	26	27	28	29	30	31	32

The 32 well cut PCR plate is marked with 'HLA-A*23' in silver/gray ink.

Well No. 1 is marked with the Lot Number '54M'.

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-A*23 SSP subtypings will be influenced three A*01, several A*02, two A*03, two A*11, most A*24, the A*25:11, two A*26, two A*29, several A*30, two A*31, three A*32, two A*33 and four A*68 alleles when present on the other haplotype. In addition, the B*18:27 allele will be amplified by primer mixes 1, 3, 10, 14 and 15, and primer mix 12 weakly amplifies the B*13:39, 38:16, 40:13, 40:19, 40:37, 40:109, 44:02:19, 44:05:03, 44:18, 44:25, 44:50, 44:75, 44:95, 49:01:01-49:01:03, 49:04-49:20, 51:103, 51:119 and 57:51 alleles.

UNIQUELY IDENTIFIED ALLELES

All the HLA-A*23 alleles, i.e. **A*23:01 to A*23:46 alleles**, recognized by the HLA Nomenclature Committee in July 2011¹ will give rise to unique amplification patterns by the primers in the HLA-A*23 subtyping kit.

The HLA-A*23 subtyping kit cannot distinguish the A*23:01:01-23:01:07 alleles or the A*23:03:01-23:03:02 alleles.

The A*23:08N and 23:22 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 7.

The A*23:09 and 23:26 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 8.

The A*23:10 and 23:23 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 10.

The A*23:13 and 23:33 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 17.

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The A*23:15 and 23:27 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 19.

The A*23:16 and 23:29 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 20.

The A*23:18 and 23:28 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 4.

The A*23:19Q and 23:31 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 22.

The A*23:36 and 23:39 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 26.

The A*23:41 and 23:42 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 25.

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

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 53 alleles generate 36 amplification patterns that can be combined in 666 homozygous and heterozygous combinations. 357 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.

++++-----	-----	-+---+---	-----	*23:03:01, *23:18 = *23:18, *23:25
+++-+---	-----	-+---+---	-----	*23:03:01, *23:06 = *23:06, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:07N = *23:07N, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:08N = *23:08N, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:09 = *23:09, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:05 = *23:05, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:10 = *23:10, *23:25 = *23:25, *23:43
+++-----	-----	-+---+---	-----	*23:03:01, *23:11N = *23:11N, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:12 = *23:12, *23:25
+++-----	-----	-+---+---	-----	*23:02, *23:03:01 = *23:02, *23:25 = *23:03:01, *23:30 = *23:24, *23:25 = *23:25, *23:30
+++-----	-----	-+---+---	-----	*23:03:01, *23:14 = *23:14, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:13 = *23:13, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:15 = *23:15, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:16 = *23:16, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:45 = *23:25, *23:45
+++-----	-----	-+---+---	-----	*23:03:01, *23:19Q = *23:19Q, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:20 = *23:20, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:40 = *23:25, *23:40
+++-----	-----	-+---+---	-----	*23:03:01, *23:21 = *23:21, *23:25
+++-----	-----	-+---+---	-----	*23:03:01, *23:41 = *23:25, *23:41
+++-----	-----	-+---+---	-----	*23:03:01, *23:36 = *23:25, *23:36
+++-----	-----	-+---+---	-----	*23:03:01, *23:35 = *23:25, *23:34 = *23:25, *23:35
+++-----	-----	-+---+---	-----	*23:03:01, *23:32 = *23:25, *23:32
+++-----	-----	-+---+---	-----	*23:03:01, *23:37 = *23:25, *23:37
+++-----	-----	-+---+---	-----	*23:03:01, *23:38N = *23:25, *23:38N
+++-----	-----	-+---+---	-----	*23:03:01, *23:44 = *23:25, *23:44
+++-----	-----	-+---+---	-----	*23:03:01, *23:46 = *23:25, *23:46
+++-----	-----	-+---+---	-----	*23:01:01, *23:03:01 = *23:01:01, *23:25 = *23:03:01, *23:17 = *23:03:01, *23:25 = *23:17, *23:25 = *23:25, *23:25
+++-----	-----	-+---+---	-----	*23:10, *23:18 = *23:18, *23:43
+++-----	-----	-+---+---	-----	*23:02, *23:18 = *23:18, *23:24 = *23:18, *23:30
+++-----	-----	-+---+---	-----	*23:18, *23:34 = *23:18, *23:35

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++----- +----- +----- +----- *23:01:01, *23:18 = *23:17, *23:18 = *23:18, *23:18
++----- +----- +----- +----- *23:06, *23:10 = *23:06, *23:43
++----- +----- +----- +----- *23:02, *23:06 = *23:06, *23:24 = *23:06, *23:30
++----- +----- +----- +----- *23:06, *23:34 = *23:06, *23:35
++----- +----- +----- +----- *23:01:01, *23:06 = *23:06, *23:06 = *23:06, *23:17
++----- +----- +----- +----- *23:07N, *23:10 = *23:07N, *23:43
++----- +----- +----- +----- *23:02, *23:07N = *23:07N, *23:24 = *23:07N, *23:30
++----- +----- +----- +----- *23:07N, *23:34 = *23:07N, *23:35
++----- +----- +----- +----- *23:01:01, *23:07N = *23:07N, *23:07N = *23:07N, *23:17
++----- +----- +----- +----- *23:08N, *23:10 = *23:08N, *23:43
++----- +----- +----- +----- *23:02, *23:08N = *23:08N, *23:24 = *23:08N, *23:30
++----- +----- +----- +----- *23:08N, *23:34 = *23:08N, *23:35
++----- +----- +----- +----- *23:01:01, *23:08N = *23:08N, *23:08N = *23:08N, *23:17
++----- +----- +----- +----- *23:09, *23:10 = *23:09, *23:43
++----- +----- +----- +----- *23:02, *23:09 = *23:09, *23:24 = *23:09, *23:30
++----- +----- +----- +----- *23:09, *23:34 = *23:09, *23:35
++----- +----- +----- +----- *23:01:01, *23:09 = *23:09, *23:09 = *23:09, *23:17
++----- +----- +----- +----- *23:05, *23:10 = *23:05, *23:43
++----- +----- +----- +----- *23:02, *23:05 = *23:05, *23:24 = *23:05, *23:30
++----- +----- +----- +----- *23:05, *23:34 = *23:05, *23:35
++----- +----- +----- +----- *23:01:01, *23:05 = *23:05, *23:05 = *23:05, *23:17
++----- +----- +----- +----- *23:10, *23:11N = *23:11N, *23:43
++----- +----- +----- +----- *23:10, *23:12 = *23:12, *23:43
++----- +----- +----- +----- *23:02, *23:10 = *23:02, *23:43 = *23:10, *23:24 = *23:10, *23:30
= *23:24, *23:43 = *23:30, *23:43
++----- +----- +----- +----- *23:10, *23:14 = *23:14, *23:43
++----- +----- +----- +----- *23:10, *23:13 = *23:13, *23:43
++----- +----- +----- +----- *23:10, *23:15 = *23:15, *23:43
++----- +----- +----- +----- *23:10, *23:16 = *23:16, *23:43
++----- +----- +----- +----- *23:10, *23:45 = *23:43, *23:45
++----- +----- +----- +----- *23:10, *23:19Q = *23:19Q, *23:43
++----- +----- +----- +----- *23:10, *23:20 = *23:20, *23:43
++----- +----- +----- +----- *23:10, *23:40 = *23:40, *23:43
++----- +----- +----- +----- *23:10, *23:21 = *23:21, *23:43
++----- +----- +----- +----- *23:10, *23:41 = *23:41, *23:43
++----- +----- +----- +----- *23:10, *23:36 = *23:36, *23:43
++----- +----- +----- +----- *23:10, *23:34 = *23:10, *23:35 = *23:34, *23:43 = *23:35, *23:43
++----- +----- +----- +----- *23:10, *23:32 = *23:32, *23:43
++----- +----- +----- +----- *23:10, *23:37 = *23:37, *23:43
++----- +----- +----- +----- *23:10, *23:38N = *23:38N, *23:43
++----- +----- +----- +----- *23:10, *23:44 = *23:43, *23:44
++----- +----- +----- +----- *23:10, *23:46 = *23:43, *23:46
++----- +----- +----- +----- *23:01:01, *23:10 = *23:01:01, *23:43 = *23:10, *23:10 = *23:10,
*23:17 = *23:10, *23:43 = *23:17, *23:43
++----- +----- +----- +----- *23:02, *23:11N = *23:11N, *23:24 = *23:11N, *23:30
++----- +----- +----- +----- *23:11N, *23:34 = *23:11N, *23:35
++----- +----- +----- +----- *23:01:01, *23:11N = *23:11N, *23:11N = *23:11N, *23:17
++----- +----- +----- +----- *23:02, *23:12 = *23:12, *23:24 = *23:12, *23:30
++----- +----- +----- +----- *23:12, *23:34 = *23:12, *23:35
++----- +----- +----- +----- *23:01:01, *23:12 = *23:12, *23:12 = *23:12, *23:17
++----- +----- +----- +----- *23:02, *23:04 = *23:04, *23:24 = *23:04, *23:30
++----- +----- +----- +----- *23:02, *23:14 = *23:14, *23:24 = *23:14, *23:30
++----- +----- +----- +----- *23:02, *23:13 = *23:13, *23:24 = *23:13, *23:30
++----- +----- +----- +----- *23:02, *23:15 = *23:15, *23:24 = *23:15, *23:30
++----- +----- +----- +----- *23:02, *23:16 = *23:16, *23:24 = *23:16, *23:30
++----- +----- +----- +----- *23:02, *23:45 = *23:24, *23:45 = *23:30, *23:45
++----- +----- +----- +----- *23:02, *23:19Q = *23:19Q, *23:24 = *23:19Q, *23:30
++----- +----- +----- +----- *23:02, *23:20 = *23:20, *23:24 = *23:20, *23:30

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++----- +----- +----- +----- *23:02, *23:40 = *23:24, *23:40 = *23:30, *23:40
++----- +----- +----- +----- *23:02, *23:21 = *23:21, *23:24 = *23:21, *23:30
++----- +----- +----- +----- *23:02, *23:41 = *23:24, *23:41 = *23:30, *23:41
++----- +----- +----- +----- *23:02, *23:36 = *23:24, *23:36 = *23:30, *23:36
++----- +----- +----- +----- *23:02, *23:34 = *23:02, *23:35 = *23:24, *23:35 = *23:30, *23:34
= *23:30, *23:35
++----- +----- +----- +----- *23:02, *23:32 = *23:24, *23:32 = *23:30, *23:32
++----- +----- +----- +----- *23:02, *23:37 = *23:24, *23:37 = *23:30, *23:37
++----- +----- +----- +----- *23:02, *23:38N = *23:24, *23:38N = *23:30, *23:38N
++----- +----- +----- +----- *23:02, *23:44 = *23:24, *23:44 = *23:30, *23:44
++----- +----- +----- +----- *23:02, *23:46 = *23:24, *23:46 = *23:30, *23:46
++----- +----- +----- +----- *23:01:01, *23:02 = *23:01:01, *23:24 = *23:01:01, *23:30 =
*23:02, *23:17 = *23:02, *23:24 = *23:02, *23:30 = *23:17, *23:24
= *23:17, *23:30 = *23:24, *23:30 = *23:30, *23:30
++----- +----- +----- +----- *23:04, *23:34 = *23:04, *23:35
++----- +----- +----- +----- *23:01:01, *23:04 = *23:04, *23:17
++----- +----- +----- +----- *23:14, *23:34 = *23:14, *23:35
++----- +----- +----- +----- *23:01:01, *23:14 = *23:14, *23:14 = *23:14, *23:17
++----- +----- +----- +----- *23:13, *23:34 = *23:13, *23:35
++----- +----- +----- +----- *23:01:01, *23:13 = *23:13, *23:13 = *23:13, *23:17
++----- +----- +----- +----- *23:15, *23:34 = *23:15, *23:35
++----- +----- +----- +----- *23:01:01, *23:15 = *23:15, *23:15 = *23:15, *23:17
++----- +----- +----- +----- *23:16, *23:34 = *23:16, *23:35
++----- +----- +----- +----- *23:01:01, *23:16 = *23:16, *23:16 = *23:16, *23:17
++----- +----- +----- +----- *23:34, *23:45 = *23:35, *23:45
++----- +----- +----- +----- *23:01:01, *23:45 = *23:17, *23:45 = *23:19Q, *23:20 = *23:19Q,
*23:45 = *23:20, *23:45 = *23:45, *23:45
++----- +----- +----- +----- *23:19Q, *23:34 = *23:19Q, *23:35
++----- +----- +----- +----- *23:01:01, *23:19Q = *23:17, *23:19Q = *23:19Q, *23:19Q
++----- +----- +----- +----- *23:20, *23:34 = *23:20, *23:35
++----- +----- +----- +----- *23:01:01, *23:20 = *23:17, *23:20 = *23:20, *23:20
++----- +----- +----- +----- *23:34, *23:40 = *23:35, *23:40
++----- +----- +----- +----- *23:21, *23:34 = *23:21, *23:35
++----- +----- +----- +----- *23:01:01, *23:40 = *23:17, *23:40 = *23:21, *23:37 = *23:21,
*23:40 = *23:37, *23:40 = *23:40, *23:40
++----- +----- +----- +----- *23:01:01, *23:21 = *23:17, *23:21 = *23:21, *23:21
++----- +----- +----- +----- *23:34, *23:41 = *23:35, *23:41
++----- +----- +----- +----- *23:01:01, *23:41 = *23:17, *23:41 = *23:41, *23:41
++----- +----- +----- +----- *23:34, *23:36 = *23:35, *23:36
++----- +----- +----- +----- *23:01:01, *23:36 = *23:17, *23:36 = *23:36, *23:36
++----- +----- +----- +----- *23:32, *23:34 = *23:32, *23:35
++----- +----- +----- +----- *23:34, *23:37 = *23:35, *23:37
++----- +----- +----- +----- *23:34, *23:38N = *23:35, *23:38N
++----- +----- +----- +----- *23:34, *23:44 = *23:35, *23:44
++----- +----- +----- +----- *23:34, *23:46 = *23:35, *23:46
++----- +----- +----- +----- *23:01:01, *23:34 = *23:01:01, *23:35 = *23:17, *23:34 = *23:17,
*23:35 = *23:34, *23:35 = *23:35, *23:35
++----- +----- +----- +----- *23:01:01, *23:32 = *23:17, *23:32 = *23:32, *23:32
++----- +----- +----- +----- *23:01:01, *23:37 = *23:17, *23:37 = *23:37, *23:37
++----- +----- +----- +----- *23:01:01, *23:38N = *23:17, *23:38N = *23:38N, *23:38N
++----- +----- +----- +----- *23:01:01, *23:44 = *23:17, *23:44 = *23:44, *23:44
++----- +----- +----- +----- *23:01:01, *23:46 = *23:17, *23:46 = *23:46, *23:46
++----- +----- +----- +----- *23:01:01, *23:01:01 = *23:01:01, *23:17

*23:01:01 = *23:01:01-23:01:07
*23:03:01 = *23:03:01-23:03:02
*23:08N = *23:08N and 23:22
*23:09 = *23:09 and 23:26

*23:10 = *23:10 and 23:23
*23:13 = *23:13 and 23:33
*23:15 = *23:15 and 23:27
*23:16 = *23:16 and 23:29

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*23:18 = *23:18 and 23:28
*23:19Q = *23:19Q and 23:31

*23:36 = *23:36 and 23:39
*23:41 = *23:41 and 23:42

SPECIFICITY TABLE

HLA-A*23 SSP subtyping

Specificities and sizes of the PCR products of the 32 primer mixes used for HLA-A*23 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified HLA-A*23 alleles ³	Other amplified HLA Class I alleles ⁴
1⁶	210 bp	800 bp	*23:01:01- 23:01:07, 23:03:01-23:46	*02:17:01-02:17:02, 02:108, 02:110, 02:268, 02:300, 02:303, 24:13:01- 24:13:02, 24:18, 24:24, 24:94, 29:07, 31:29, B*18:27
2	160 bp	1070 bp	*23:01:01-23:02, 23:05-23:42, 23:44-23:46	*02:19, 02:36-02:37, 02:54, 02:255, 24:02:01:01-24:02:41, 24:02:43- 24:02:51, 24:04-24:09N, 24:11N, 24:13:01-24:15, 24:17, 24:19-24:20, 24:24-24:32, 24:34-24:64, 24:66- 24:74, 24:76-24:93, 24:95-24:109, 24:111-24:124, 24:126-24:137, 24:139-24:157, 24:159-24:166, 24:168-24:182, 33:19, 68:26, 68:65
3^{5,8}	125 bp, 270 bp	800 bp	*23:03:01- 23:03:02, 23:25	*24:21:01, 29:07, 31:29, B*18:27
4^{5,9}	90 bp, 200 bp	1070 bp	*23:18, 23:28	*24:77
5	230 bp	800 bp	*23:06	*31:36
6	470 bp	1070 bp	*23:07N	*01:04N, 03:21N, 11:21N, 24:11N
7^{5,10}	95 bp, 205 bp	800 bp	*23:08N, 23:22	*02:82N
8^{7,11}	135 bp, 215 bp	1070 bp	*23:09, 23:26	*01:02, 01:20, 24:129
9	235 bp	1070 bp	*23:05	*24:25
10¹²	135 bp, 235 bp	800 bp	*23:10, 23:23, 23:43	*24:10 ^W , 24:46, B*18:27
11	200 bp	800 bp	*23:11N	
12	190 bp	1070 bp	*23:12	*24:30, 24:42, 25:11, 32:08, B*13:39^W, 38:16^W, 40:13^W, 40:19^W, 40:37^W, 40:109^W, 44:02:19^W, 44:05:03^W, 44:18^W, 44:25^W, 44:50^W, 44:75^W, 44:95^W, 49:01:01^W- 49:01:03^W, 49:04^W-49:20^W, 51:103^W, 51:119^W, 57:51^W

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Lot-specific information

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13^{5,13}	90 bp, 210 bp	800 bp	*23:02, 23:24, 23:30	*24:06, 24:87, 24:138, 24:167
14	245 bp	1070 bp	*23:04	*02:17:01-02:17:02, 02:108, 02:110, 02:268, 02:300, 02:303, 24:03:01- 24:03:02, 24:10, 24:18, 24:22, 24:33, 24:94, 24:125, 24:138, 24:167, 29:07, 31:29, B*18:27
15	210 bp	1070 bp	*23:14	*24:13:02, B*18:27
16¹⁴	175 bp, 205 bp	800 bp	*23:14	*02:17:01 ^w -02:17:02 ^w , 24:02:01:01- 24:11N, 24:13:01-24:13:02, 24:17- 24:50, 24:54-24:56, 24:58-24:63, 24:66-24:91, 24:93, 24:95-24:113, 24:115-24:137, 24:139-24:182, 26:16, 33:19, 68:45
17¹⁵	125 bp, 225 bp	1070 bp	*23:13, 23:33	*03:72, 11:88, 24:07, 24:19, 24:24, 24:131, 30:01:01-30:01:05, 30:11:01-30:11:02, 30:14L-30:20, 30:23-30:26, 30:30-30:31, 30:35- 30:43, 30:48-30:49, 30:52-30:54, 68:45
18⁵	110 bp	1070 bp	*23:01:01- 23:01:07, 23:02 ^w , 23:04-23:13, 23:14 ^w , 23:15- 23:23, 23:25- 23:33, 23:35-23:46	*02:40, 02:51, 02:130, 24:24, 32:28, 33:32, 68:51 ^w
19^{5,16}	120 bp, 260 bp	800 bp	*23:15, 23:27	
20¹⁷	130 bp, 230 bp	800 bp	*23:16, 23:29	*24:128
21⁵	90 bp	800 bp	*23:01:01- 23:01:07, 23:02 [?] - 23:04 [?] , 23:06- 23:07N, 23:08N [?] , 23:09, 23:10 [?] - 23:16 [?] , 23:18 [?] - 23:25 [?] , 23:26, 23:27 [?] -23:37 [?] , 23:38N, 23:39 [?] - 23:46 [?]	
22^{5,6,18}	90 bp, 290 bp	1070 bp	*23:19Q, 23:31, 23:45	*24:17, 24:41, 24:62, 24:106, 29:07, 31:29
23^{5,7,19}	80 bp, 170 bp	1070 bp	*23:20, 23:45	*02:41, 02:80, 02:117, 02:289, 02:304, 24:62, 26:10, 32:28, 33:32
24²⁰	180 bp, 240 bp	800 bp	*23:21, 23:40	

25 ^{5,21}	95 bp, 205 bp	1070 bp	*23:41-23:42	*02:221, 29:15
26 ^{6,22}	145 bp, 245 bp	1070 bp	*23:36, 23:39	*24:32, 30:22, 32:07
27 ²³	205 bp, 285 bp	1070 bp	*23:34-23:35	
28	270 bp	1070 bp	*23:32	
29 ²⁴	200 bp, 240 bp	1070 bp	*23:37, 23:40	
30	245 bp	1070 bp	*23:38N	*24:61
31	185 bp	1070 bp	*23:44	
32	195 bp	1070 bp	*23:46	*24:66

¹ Alleles 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-A*23 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.

² The 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-A*23 subtyping.

In addition, wells number 3, 5, 7, 10, 11, 13, 16, 19 to 21 and 24 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.

³ For several HLA-A alleles 1st or 4th 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 these regions are conserved within allelic groups.

The A*23:08N and 23:22 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 7.

The A*23:09 and 23:26 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 8.

The A*23:10 and 23:23 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 10.

The A*23:13 and 23:33 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 17.

The A*23:15 and 23:27 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 19.

The A*23:16 and 23:29 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 20.

The A*23:18 and 23:28 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 4.

The A*23:19Q and 23:31 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 22.

The A*23:36 and 23:39 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 26.

The A*23:41 and 23:42 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 25.

⁴Due to the sharing of sequence motifs between HLA-A alleles non-HLA-A*23 alleles will be amplified by primer mixes 1 to 10, 12 to 18, 20, 22, 23, 25, 26, 30 and 32. In addition, the B*18:27 allele will be amplified by primer mixes 1, 3, 10, 14 and 15, and primer mix 12 weakly amplifies the B*13:39, 38:16, 40:13, 40:19, 40:37, 40:109, 44:02:19, 44:05:03, 44:18, 44:25, 44:50, 44:75, 44:95, 49:01:01-49:01:03, 49:04-49:20, 51:103, 51:119 and 57:51 alleles.

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

⁶Primer mixes 1, 22 and 26 may yield less PCR product than the other A*23 primer mixes.

⁷Primer mix 8 and 23 may give rise to nonspecific amplifications.

⁸Primer mix 3: Specific PCR fragment of 125 bp in the A*23:03:01-23:03:02 and the A*24:21:01, 29:07 and 31:29 and the B*18:27 alleles. Specific PCR fragment of 270 bp in the A*23:25 allele.

⁹Primer mix 4: Specific PCR fragment of 90 bp in the A*23:18 and 23:28 and the A*24:77 alleles. Specific PCR fragment of 200 bp in the A*23:28 and the A*24:77 alleles.

¹⁰Primer mix 7: Specific PCR fragment of 95 bp in the A*23:08N and the A*02:82N alleles. Specific PCR fragment of 205 bp in the A*23:22 allele.

¹¹Primer mix 8: Specific PCR fragment of 135 bp in the A*23:26 allele. Specific PCR fragment of 215 bp in the A*23:09 and the A*01:02, 01:20 and 24:129 alleles.

¹²Primer mix 10: Specific PCR fragment of 135 bp in the A*23:23 and in the B*18:27 alleles. Specific PCR fragment of 235 bp in the A*23:10 and 23:43 and in the A*24:10^w and 24:46 alleles.

¹³Primer mix 13: Specific PCR fragment of 90 bp in the A*23:30 allele. Specific PCR fragment of 210 bp in the A*23:02 and 23:24 and in the A*24:06, 24:87, 24:138 and 24:167 alleles.

¹⁴Primer mix 16: Specific PCR fragment of 175 bp in the A*23:14 and the A*24:02:16, 24:06, 24:13:01-24:13:02, 24:18, 24:22, 24:54, 24:87, 24:88, 24:91, 24:107, 24:133 and 24:167 alleles. Specific PCR fragment of 205 bp in the A*24:05, 24:24, 24:67, 24:130, 24:168, 26:16 and 68:45 alleles. Specific PCR fragment of 175 and 205 bp in the A*24:02:01:01-24:02:15, 24:02:17-24:04, 24:07-24:11N, 24:17, 24:19-24:21:02, 24:23, 24:25-24:50, 24:55-24:56, 24:58-24:63, 24:66-24:86N, 24:88-24:90N, 24:93, 24:95-24:106, 24:108-24:113, 24:115-24:129, 24:131-24:132N, 24:134-24:137, 24:139-24:166, 24:169-24:182 and 33:19 alleles. Both fragments may not always be visible.

¹⁵Primer mix 17: Specific PCR fragment of 125 bp in the A*23:33 allele. Specific PCR fragment of 225 bp in the A*23:13 and the A*03:72, 11:88, 24:07, 24:19, 24:24, 24:131, 30:01:01-30:01:05, 30:11:01-30:11:02, 30:14L-30:20, 30:23-30:26, 30:30-30:31, 30:35-30:43, 30:48-30:49, 30:52-30:54 and 68:45 alleles.

¹⁶Primer mix 19: Specific PCR fragment of 120 bp in the A*23:15 allele. Specific PCR fragment of 260 bp in the A*23:27 allele.

¹⁷Primer mix 20: Specific PCR fragment of 130 bp in the A*23:29 and in the A*24:128 alleles. Specific PCR fragment of 230 bp in the A*23:16 allele.

¹⁸Primer mix 22: Specific PCR fragment of 90 bp in the A*23:31 and 23:45 and the A*24:17, 24:41, 24:62, 24:106, 29:07 and 31:29 alleles. Specific PCR fragment of 290 bp in the A*23:19Q allele.

¹⁹Primer mix 23: Specific PCR fragment of 80 bp in the A*23:45 and the A*02:41, 02:80, 02:117, 02:289, 02:304, 24:62, 26:10, 32:28 and 33:32 alleles. Specific PCR fragment of 170 bp in the A*23:20 allele.

²⁰Primer mix 24: Specific PCR fragment of 180 bp in the A*23:21 allele. Specific PCR fragment of 240 bp in the A*23:40 allele.

²¹Primer mix 25: Specific PCR fragment of 95 bp in the A*23:41 and the A*02:221 and 29:15 alleles. Specific PCR fragment of 205 bp in the A*23:42 allele.

²²Primer mix 26: Specific PCR fragment of 145 bp in the A*23:39 and the A*30:22 alleles. Specific PCR fragment of 245 bp in the A*23:36 and the A*24:32 and 32:07 alleles.

²³Primer mix 27: Specific PCR fragment of 205 bp in the A*23:34 allele. Specific PCR fragment of 285 bp in the A*23:35 allele.

²⁴Primer mix 29: Specific PCR fragment of 200 bp in the A*23:37 allele. Specific PCR fragment of 240 bp in the A*23:40 allele.

'w', may be weakly amplified.

'?', nucleotide sequence information not available for the primer matching sequence.

INTERPRETATION TABLE

HLA-A*23 SSP subtyping

Amplification patterns of the A*23:01 to A*23:46 alleles

	Well ¹⁴															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length of spec. PCR product(s)	210	160	125	90	230	470	95	135	235	135	200	190	90	245	210	175
Length of int. pos. control ¹	800	1070	800	1070	800	1070	800	1070	1070	800	800	1070	800	1070	1070	800
5'-primer(s) ²	368	453	368	98	144	374	98	98	28	368	160	144	368	368	368	98
	5'-gTT 3'	5'-AAA 3'	5'-gTT 3'	5'-CTC 3'	5'-gCC 3'	5'-ATA 3'	5'-CTC 3'	5'-CTC 3'	5'-TCg 3'	5'-gTT 3'	5'-ACg 3'	5'-gCC 3'	5'-gTT 3'	5'-gTT 3'	5'-gTT 3'	5'-CTC 3'
				678			564									368
				5'-Aga 3'			5'-Tga 3'									5'-gTT 3'
3'-primer(s) ³	539	570	453	256	331	621	262	193	92	463	317	292	419	570	538	259
	5'-TCA 3'	5'-CCg 3'	5'-TCg 3'	5'-CTg 3'	5'-CTC 3'	5'-ggg 3'	5'-TgC 3'	5'-CgA 3'	5'-AAC 3'	5'-gCT 3'	5'-ggA 3'	5'-gTg 3'	5'-CgC 3'	5'-CAC 3'	5'-Cag 3'	5'-gTT 3'
			595	728			616	271		559			530			502
			5'-CCg 3'	5'-CCT 3'			5'-CgT 3'	5'-CAT 3'		5'-CCg 3'			5'-CCA 3'			5'-CTT 3'
										571			539			539
										5'-CCT 3'			5'-TCC 3'			5'-TCT 3'
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
HLA-A allele																
*23:01:01-23:01:07	1	2														
*23:02		2											13			
*23:03:01-23:03:02	1		3													
*23:04	1													14		
*23:05	1	2							9							
*23:06	1	2			5											
*23:07N	1	2				6										
*23:08N, 23:22 ⁴	1	2					7									
*23:09, 23:26 ⁵	1	2						8								
*23:10, 23:23 ⁶	1	2								10						
*23:11N	1	2									11					
*23:12	1	2										12				
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

INTERPRETATION TABLE																
HLA-A*23 SSP subtyping																
Amplification patterns of the A*23:01 to A*23:46 alleles																
Well¹⁴																
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
125	110	120	130	90	90	80	180	95	145	205	270	200	245	185	195	Length of spec. PCR product(s)
225		260	230		290	170	240	205	245	285		240				Length of int. pos. control ¹
1070	1070	800	800	800	1070	1070	800	1070	1070	1070	1070	1070	1070	1070	1070	5'-primer(s) ²
98	453	98	228	920	368	414	98	376	98	368	90	98	98	395	418	3'-primer(s) ³
5'-CTC 3'	5'-AAA 3'	5'-CTC 3'	5'-ATg 3'	5'-CCA 3'	5'-gTT 3'	5'-Cag 3'	5'-CTC 3'	5'-gCT 3'	5'-CTC 3'	5'-gTT 3'	5'-AgT 3'	5'-CTC 3'	5'-CTC 3'	5'-gCA 3'	5'-AgC 3'	
		493	379			678		484								
		5'-CTg 3'	5'-ACA 3'			5'-AgA 3'		5'-ACg 3'								
181	524	316	317	971	418	453	238	538	200	534	317	255	303	538	570	
5'-gTA 3'	5'-CAC 3'	5'-gAA 3'	5'-gGA 3'	5'-Cag 3'	5'-gTC 3'	5'-TCT 3'	5'-CCT 3'	5'-CAA 3'	5'-TCC 3'	5'-CgT 3'	5'-gGA 3'	5'-TCC 3'	5'-AgT 3'	5'-CAA 3'	5'-CCg 3'	
282		570	570		619	806	299		302	614		299				
5'-gAC 3'		5'-CCg 3'	5'-CCg 3'		5'-gTT 3'	5'-CTA 3'	5'-TCg 3'		5'-ggC 3'	5'-Tgg 3'		5'-TCg 3'				
282																
5'-gAC 3'																
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.
																HLA-A allele
	18			21												*23:01:01-23:01:07
	w			?												*23:02
				?												*23:03:01-23:03:02
	18			?												*23:04
	18															*23:05
	18			21												*23:06
	18			21												*23:07N
	18			?												*23:08N, 23:22 ⁴
	18			21												*23:09, 23:26 ⁵
	18			?												*23:10, 23:23 ⁶
	18			?												*23:11N
	18			?												*23:12
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.

Lot No.: **54M**

Lot-specific information

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Length of spec.	210	160	125	90	230	470	95	135	235	135	200	190	90	245	210	175
PCR product(s)			270	200			205	215		235			210			205
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*23:13, 23:33 ⁷	1	2														
*23:14	1	2													15	16
*23:14	1	2														16
*23:15, 23:27 ⁸	1	2														
*23:16, 23:29 ⁹	1	2														
*23:17	1	2														
*23:18, 23:28 ¹⁰	1	2		4												
*23:19Q, 23:31 ¹¹	1	2														
*23:20	1	2														
*23:21	1	2														
*23:24	1	2											13			
*23:25	1	2	3													
*23:30	1	2											13			
*23:32	1	2														
*23:34	1	2														
*23:35	1	2														
*23:36, 23:39 ¹²	1	2														
*23:37	1	2														
*23:37	1	2														
*23:38N	1	2														
*23:40	1	2														
*23:41, 23:42 ¹³	1	2														
*23:43	1									10						
*23:44	1	2														
*23:45	1	2														
*23:46	1	2														
*01:02, 01:20								8								
*01:04N, 03:21N, 11:21N						6										
*02:17:01-02:17:02	1													14		w
*02:19, 02:36-02:37, 02:54, 02:255, 24:14-24:15, 24:51- 24:53, 24:57, 24:64, 24:92, 24:114, 68:26, 68:65		2														
*02:40, 02:51, 02:130																
*02:41, 02:80, 02:117, 02:289, 02:304, 26:10																
*02:82N							7									
*02:108, 02:110, 02:268, 02:300, 02:303, 24:94	1													14		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Lot No.: **54M**

Lot-specific information

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125	110	120	130	90	90	80	180	95	145	205	270	200	245	185	195	Length of spec.
225		260	230		290	170	240	205	245	285		240				PCR product(s)
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.
17	18			?												*23:13, 23:33 ⁷
	w			?												*23:14
	18			?												*23:14
	18	19		?												*23:15, 23:27 ⁸
	18		20	?												*23:16, 23:29 ⁹
	18															*23:17
	18			?												*23:18, 23:28 ¹⁰
	18			?	22											*23:19Q, 23:31 ¹¹
	18			?		23										*23:20
	18			?			24									*23:21
				?												*23:24
	18			?												*23:25
	18			?												*23:30
	18			?							28					*23:32
				?						27						*23:34
	18			?						27						*23:35
	18			?				26								*23:36, 23:39 ¹²
	18			?								29				*23:37
	18			?												*23:37
	18			21									30			*23:38N
	18			?		24						29				*23:40
	18			?				25								*23:41, 23:42 ¹³
	18			?												*23:43
	18			?										31		*23:44
	18			?	22	23										*23:45
	18			?											32	*23:46
																*01:02, 01:20
																*01:04N, 03:21N, 11:21N
																*02:17:01-02:17:02
																*02:19, 02:36-02:37, 02:54, 02:255, 24:14-24:15, 24:51- 24:53, 24:57, 24:64, 24:92, 24:114, 68:26, 68:65
	18															*02:40, 02:51, 02:130
						23										*02:41, 02:80, 02:117, 02:289, 02:304, 26:10
																*02:82N
																*02:108, 02:110, 02:268, 02:300, 02:303, 24:94
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.

Lot No.: **54M**

Lot-specific information

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Length of spec.	210	160	125	90	230	470	95	135	235	135	200	190	90	245	210	175
PCR product(s)			270	200			205	215		235			210			205
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*02:221, 29:15																
*03:72, 11:88, 30:01:01-30:01:05, 30:11:01-30:11:02, 30:14L-30:20, 30:23-30:26, 30:30-30:31, 30:35-30:43, 30:48-30:49, 30:52-30:54																
*24:02:01:01-24:02:41, 24:02:43-24:02:51, 24:04-24:05, 24:08-24:09N, 24:20, 24:26-24:29, 24:31, 24:34-24:40N, 24:43-24:45N, 24:47-24:50, 24:54-24:56, 24:58-24:60N, 24:63, 24:67-24:74, 24:76, 24:78-24:86N, 24:88-24:91, 24:93, 24:95-24:105, 24:107-24:109, 24:111-24:113, 24:115-24:124, 24:126-24:127, 24:130, 24:132N-24:137, 24:139-24:157, 24:159-24:166, 24:168-24:182, 33:19		2														16
*24:02:42, 24:21:02, 24:23, 24:75, 24:110, 24:158N, 26:16																16
*24:03:01-24:03:02, 24:22, 24:33, 24:125														14		16
*24:06, 24:87		2											13			16
*24:07, 24:19, 24:131		2														16
*24:10										w				14		16
*24:11N		2				6										16
*24:13:01	1	2														16
*24:13:02	1	2													15	16
*24:17, 24:41, 24:106		2														16
*24:18	1													14		16
*24:21:01			3													16
*24:24	1	2														16
*24:25		2							9							16
*24:30, 24:42		2										12				16
*24:32		2														16
*24:46		2								10						16
*24:61		2														16
*24:62		2														16
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Lot No.: **54M**

Lot-specific information

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125	110	120	130	90	90	80	180	95	145	205	270	200	245	185	195	Length of spec.
225		260	230		290	170	240	205	245	285		240				PCR product(s)
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.
								25								*02:221, 29:15
17																*03:72, 11:88, 30:01:01-30:01:05, 30:11:01-30:11:02, 30:14L-30:20, 30:23-30:26, 30:30-30:31, 30:35-30:43, 30:48-30:49, 30:52-30:54
																*24:02:01:01-24:02:41, 24:02:43-24:02:51, 24:04-24:05, 24:08-24:09N, 24:20, 24:26-24:29, 24:31, 24:34-24:40N, 24:43-24:45N, 24:47-24:50, 24:54-24:56, 24:58-24:60N, 24:63, 24:67-24:74, 24:76, 24:78-24:86N, 24:88-24:91, 24:93, 24:95-24:105, 24:107-24:109, 24:111-24:113, 24:115-24:124, 24:126-24:127, 24:130, 24:132N-24:137, 24:139-24:157, 24:159-24:166, 24:168-24:182, 33:19
																*24:02:42, 24:21:02, 24:23, 24:75, 24:110, 24:158N, 26:16
																*24:03:01-24:03:02, 24:22, 24:33, 24:125
17																*24:06, 24:87
																*24:07, 24:19, 24:131
																*24:10
																*24:11N
																*24:13:01
																*24:13:02
					22											*24:17, 24:41, 24:106
																*24:18
17	18															*24:21:01
																*24:24
																*24:25
																*24:30, 24:42
								26								*24:32
																*24:46
													30			*24:61
					22	23										*24:62
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.

Lot No.: **54M**

Lot-specific information

www.olerup-ssp.com

Length of spec.	210	160	125	90	230	470	95	135	235	135	200	190	90	245	210	175
PCR product(s)			270	200			205	215		235			210			205
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*24:66		2														16
*24:77		2		4												16
*24:128		2														16
*24:129		2						8								16
*24:138													13	14		
*24:167													13	14		16
*25:11, 32:08												12				
*29:07, 31:29	1		3											14		
*30:22, 32:07																
*31:36					5											
*32:28, 33:32																
*68:45																16
*68:51																
B*13:39, 38:16, 40:13, 40:19, 40:37, 40:109, 44:02:19, 44:05:03, 44:18, 44:25, 44:50, 44:75, 44:95, 49:01:01- 49:01:03, 49:04-49:20, 51:103, 51:119, 57:51														w		
B*18:27	1		3							10				14	15	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

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-A*23 subtyping. .

In addition, wells number 3, 5, 7, 10, 11, 13, 16, 19 to 21 and 24 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 exons or 3rd 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, 3rd or 4th exons, 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 A*23:08N and 23:22 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 7.

⁵The A*23:09 and 23:26 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 8.

⁶The A*23:10 and 23:23 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 10.

⁷The A*23:13 and 23:33 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 17.

⁸The A*23:15 and 23:27 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 19.

Lot No.: **54M**

Lot-specific information

www.olerup-ssp.com

125	110	120	130	90	90	80	180	95	145	205	270	200	245	185	195	Length of spec. PCR product(s)	
225		260	230		290	170	240	205	245	285		240				Well No.	
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.	
															32	*24:66	
																*24:77	
			20													*24:128	
																*24:129	
																*24:138	
																*24:167	
																*25:11, 32:08	
					22											*29:07, 31:29	
									26							*30:22, 32:07	
																*31:36	
	18					23										*32:28, 33:32	
17																*68:45	
	w															*68:51	
																<i>B*13:39, 38:16, 40:13, 40:19, 40:37, 40:109, 44:02:19, 44:05:03, 44:18, 44:25, 44:50, 44:75, 44:95, 49:01:01- 49:01:03, 49:04-49:20, 51:103, 51:119, 57:51</i>	
																<i>B*18:27</i>	
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Well No.	

⁹The A*23:16 and 23:29 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 20.

¹⁰The A*23:18 and 23:28 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 4.

¹¹The A*23:19Q and 23:31 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 22.

¹²The A*23:36 and 23:39 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 26.

¹³The A*23:41 and 23:42 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 25.

¹⁴Primer mix 3: Specific PCR fragment of 125 bp in the A*23:03:01-23:03:02 and the A*24:21:01, 29:07 and 31:29 and the B*18:27 alleles. Specific PCR fragment of 270 bp in the A*23:25 allele.

Primer mix 4: Specific PCR fragment of 90 bp in the A*23:18 and 23:28 and the A*24:77 alleles. Specific PCR fragment of 200 bp in the A*23:28 and the A*24:77 alleles.

Primer mix 7: Specific PCR fragment of 95 bp in the A*23:08N and the A*02:82N alleles. Specific PCR fragment of 205 bp in the A*23:22 allele.

Primer mix 8: Specific PCR fragment of 135 bp in the A*23:26 allele. Specific PCR fragment of 215 bp in the A*23:09 and the A*01:02, 01:20 and 24:129 alleles.

Primer mix 10: Specific PCR fragment of 135 bp in the A*23:23 and in the B*18:27 alleles. Specific PCR fragment of 235 bp in the A*23:10 and 23:43 and in the A*24:10^w and 24:46 alleles.

Primer mix 13: Specific PCR fragment of 90 bp in the A*23:30 allele. Specific PCR fragment of 210 bp in the A*23:02 and 23:24 and in the A*24:06, 24:87, 24:138 and 24:167 alleles.

Primer mix 16: Specific PCR fragment of 175 bp in the A*23:14 and the A*24:02:16, 24:06, 24:13:01-24:13:02, 24:18, 24:22, 24:54, 24:87, 24:88, 24:91, 24:107, 24:133 and 24:167 alleles. Specific PCR

fragment of 205 bp in the A*24:05, 24:24, 24:67, 24:130, 24:168, 26:16 and 68:45 alleles. Specific PCR fragment of 175 and 205 bp in the A*24:02:01:01-24:02:15, 24:02.17-24:04, 24:07-24:11N, 24:17, 24:19-24:21:02, 24:23, 24:25-24:50, 24:55-24:56, 24:58-24:63, 24:66-24:86N, 24:88-24:90N, 24:93, 24:95-24:106, 24:108-24:113, 24:115-24:129, 24:131-24:132N, 24:134-24:137, 24:139-24:166, 24:169-24:182 and 33:19 alleles. Both fragments may not always be visible.

Primer mix 17: Specific PCR fragment of 125 bp in the A*23:33 allele. Specific PCR fragment of 225 bp in the A*23:13 and the A*03:72, 11:88, 24:07, 24:19, 24:24, 24:131, 30:01:01-30:01:05, 30:11:01-30:11:02, 30:14L-30:20, 30:23-30:26, 30:30-30:31, 30:35-30:43, 30:48-30:49, 30:52-30:54 and 68:45 alleles.

Primer mix 19: Specific PCR fragment of 120 bp in the A*23:15 allele. Specific PCR fragment of 260 bp in the A*23:27 allele.

Primer mix 20: Specific PCR fragment of 130 bp in the A*23:29 and in the A* 24:128 alleles. Specific PCR fragment of 230 bp in the A*23:16 allele.

Primer mix 22: Specific PCR fragment of 90 bp in the A*23:31 and 23:45 and the A*24:17, 24:41, 24:62, 24:106, 29:07 and 31:29 alleles. Specific PCR fragment of 290 bp in the A*23:19Q allele.

Primer mix 23: Specific PCR fragment of 80 bp in the A*23:45 and the A*02:41, 02:80, 02:117, 02:289, 02:304, 24:62, 26:10, 32:28 and 33:32 alleles. Specific PCR fragment of 170 bp in the A*23:20 allele.

Primer mix 24: Specific PCR fragment of 180 bp in the A*23:21 allele. Specific PCR fragment of 240 bp in the A*23:40 allele.

Primer mix 25: Specific PCR fragment of 95 bp in the A*23:41 and the A*02:221 and 29:15 alleles. Specific PCR fragment of 205 bp in the A*23:42 allele.

Primer mix 26: Specific PCR fragment of 145 bp in the A*23:39 and the A*30:22 alleles. Specific PCR fragment of 245 bp in the A*23:36 and the A*24:32 and 32:07 alleles.

Primer mix 27: Specific PCR fragment of 205 bp in the A*23:34 allele. Specific PCR fragment of 285 bp in the A*23:35 allele.

Primer mix 29: Specific PCR fragment of 200 bp in the A*23:37 allele. Specific PCR fragment of 240 bp in the A*23:40 allele.

'w', may be weakly amplified.

'?', nucleotide sequence information not available for the primer matching sequence.

CELL LINE VALIDATION SHEET																				
HLA-A*23 SSP subtyping kit																				
				Lot No.:	Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
					201189701	201189702	201189703	201189704	201189705	201189706	201189707	201189708	201189709	201189710	201189711	201189712	201189713	201189714	201189715	201189716
	IHWC cell line	A*	A*																	
1	9001 SA	*24:02			-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
2	9280 LK707	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324	*01:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373	*30:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*01:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM	*02:01	*26:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL	*26:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025 DEU	*31:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*26:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3	*24:02			-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
11	9051 PITOUT	*29:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*31:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*24:02			-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
16	9037 SWEIG007	*29:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540	*03:01	*80:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*33:03	*74:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*02:17			+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	W
22	9056 KOSE	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*02:01	*34:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*32:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*33:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*02:05			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*24:10	*29:01		-	-	-	-	-	-	-	-	W	-	-	-	+	-	+	-
28	9320 BEL5GB	*02:01	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*29:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*30:01	*68:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*30:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*31:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*31:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*32:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*02:06	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*02:17			+	-	-	-	-	-	-	-	-	-	-	-	+	-	W	-
39	9315 CML	*01:01	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*02:07	*30:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*02:07			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*02:06	*02:07		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*66:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*23:01	*24:02		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
46	9013 SCHU	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*02:16	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND	*02:01	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CELL LINE VALIDATION SHEET																					
HLA-A*23 SSP subtyping kit																					
				Lot No.:	Well																
					17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
					201189717	201189718	201189719	201189720	201189721	201189722	201189723	201189724	201189725	201189726	201189727	201189728	201189729	201189730	201189731	201189732	
	IHWC cell line	A*	A*																		
1	9001 SA	*24:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324	*01:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373	*30:01			+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*01:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM	*02:01	*26:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL	*26:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025 DEU	*31:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*26:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3	*24:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT	*29:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*31:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*24:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*29:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540	*03:01	*80:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*33:03	*74:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*02:17			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*02:01	*34:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*32:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*33:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*02:05			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*24:10	*29:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB	*02:01	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*29:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*30:01	*68:02		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*30:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*31:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*31:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*32:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*02:06	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*02:17			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML	*01:01	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*02:07	*30:01		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*02:07			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*02:06	*02:07		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*66:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*23:01	*24:02		-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*02:16	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND	*02:01	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® HLA-A*23 SSP

Product number: 101.421-06u – without *Taq* polymerase
Lot number: 54M
Expiry date: 2014-March-01
Number of tests: 6
Number of wells per test: 32

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2011-897-01	13	2011-897-13	25	2011-897-17
2	2011-897-02	14	2011-897-14	26	2011-897-18
3	2011-897-03	15	2011-897-15	27	2011-897-19
4	2011-897-04	16	2011-897-16	28	2011-897-20
5	2011-897-05	17	2011-897-17	29	2011-897-21
6	2011-897-06	18	2011-897-18	30	2011-897-22
7	2011-897-07	19	2011-897-19	31	2011-897-23
8	2011-897-08	20	2011-897-20	32	2011-897-24
9	2011-897-09	21	2011-897-21		
10	2011-897-10	22	2011-897-22		
11	2011-897-11	23	2011-897-23		
12	2011-897-12	24	2011-897-24		

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 3 to 5, 9, 11, 13, 15, 19, 20 and 23 to 32 were available. The specificities of the primers in primer solutions 3, 13, 15, 19, 23, 26 and 32 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 4, 5, 9, 24, 27, 29 and 30 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 11, 20, 25, 28 and 31 it was only possible to test the 3'-primers, the 5'-primers was not possible to test. In primer solutions 3, 7, 8, 10, 13, 17, 19, 22 and 23 one or two 3'-primers were not possible to test, and in primer solutions 19 and 23 one 5'-primer was not possible to test. Additional primers in primer solutions 7, 10 and 16 were tested by separately adding one additional 5'-primer and/or one additional 3'-primer.

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

Date of approval: 2011-November-14

Approved by:

Production Quality Control

Declaration of Conformity

Product name: *Olerup* SSP® HLA-A*23
Product number: 101.421-06u
Lot number: 54M

Intended use: HLA-A*23 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-14

Ann-Cathrin Jareman
Head of QA and Regulatory Affairs

Lot No.: **54M**

Lot-specific information

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