

Olerup SSP® DRB1*15

Product number:	101.125-24/06 – including <i>Taq</i> pol.
Lot number:	09K
Expiry date:	2012-April-01
Number of tests:	24 test – Product No. 101.125-24 6 tests – Product No. 101.125-06
Number of wells per test:	30
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. 09K.

**CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*®
DRB1*15 Lot**

The DRB1*15 specificity and interpretation tables have been updated for the DRB1 alleles described since the previous *Olerup SSP*® DRB1*15 lot was made (Lot No. 55F).

Six wells has been added to the DRB1*15 kit,
wells **25** to **30**.

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

Well	5'-primer	3'-primer	rationale
8	-	Added	New primer for the DRB1*15:36 allele.
13	-	Added	New primer for the DRB1*15:32 allele.
19	-	Added	New primer for the DRB1*15:37 allele.
22	Added	-	New primer for the DRB1*15:40 allele.
24	Added	-	New primers for the DRB1*15:40 and DRB1*15:43 alleles.
25	New	New	New primer pairs for the DRB1*15:33 and DRB1*15:39 alleles.
26	New	New	New primer pair for the DRB1*15:38 allele.
27	New	New	New primer pair for the DRB1*15:42 allele.

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28	New	New	New primer pair for the DRB1*15:35 allele.
29	New	New	New primer pair for the DRB1*15:41 allele.
30	New	New	New primer pair for the DRB1*15:44 allele.

PRODUCT DESCRIPTION

DRB1*15 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*15:01 to DRB1*15:44 alleles.

PLATE LAYOUT

Each test consists of 30 PCR reactions in a 32 well cut PCR plate. Wells 31 and 32 are 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	empty	empty

The 32 well cut PCR plate is marked with 'DRB1*15' in silver/gray ink.

Well No. 1 is marked with the Lot No. '09K'.

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

Only the DRB1*15 and DRB1*16 alleles will be amplified by the DRB1*15 subtyping kit, except that the DRB1* 04:73, 11:82, 13:09 and 14:37 alleles will be amplified by primer mix 3, and the DRB1*11:88 allele will be amplified by primer mix 12. Thus, the interpretation of DRB1*15 subtypings is only influenced by these alleles when present on the other haplotype, and not by other groups of DRB1 alleles or other DRB genes.

UNIQUELY IDENTIFIED ALLELES

All the DRB1*15 alleles, i.e. **DRB1*15:01 to DRB1*15:44**, recognized by the HLA Nomenclature Committee in April 2010¹ will give rise to unique amplification patterns by the primers in the DRB1*15 subtyping kit.

The DRB1*15 subtyping kit cannot distinguish the DRB1* 15:01:01:01 to 15:01:12 alleles, the DRB1*15:02:01 to DRB1*15:02:07 alleles or the DRB1*15:03:01:01 and DRB1*15:03:01:02 alleles.

¹DRB1 alleles listed on the IMGT/HLA web page 2010-April-01, release 3.0.0, www.ebi.ac.uk/imgt/hla.

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Resolution in homo- and heterozygotes

The 44 DRB1*15 alleles can be combined in 990 homozygous and heterozygous combinations. 332 of these genotypes do not give rise to unique amplification patterns. The different sizes of the specific PCR products generated by primer mixes 6, 8, 9, 13, 16, 19, 20, 22, 23 and 25 were not considered in these calculations.

+++++++	-----	-----	-----	15:05,15:14 = 15:14,15:36
++++++-	-----+	-----	-----	15:13,15:14 = 15:14,15:17N
++++++-	-----	-----+	-----	15:02,15:03 = 15:03,15:14
+++++++	-----	-----+	-----	15:01,15:34 = 15:07,15:27 =
				15:22,15:34 = 15:34,15:36
+++++++	-----	-----	-----	15:05,15:11 = 15:07,15:31 =
				15:11,15:36
++++++-	+-----	-----	-----	15:06,15:11 = 15:07,15:19
++++++-	-+-----	-----	-----	15:04,15:11 = 15:07,15:15
++++++-	-----+	-----	-----	15:11,15:13 = 15:11,15:17N
++++++-	-----+	-----	-----	15:07,15:08 = 15:11,15:16
++++++-	-----	-----+	-----	15:07,15:30 = 15:11,15:37
++++++-	-----	-----+	-----	15:07,15:26 = 15:11,15:43
++++++-	-----	-----	+-----	15:07,15:39 = 15:11,15:33
++++++-	-----	-----	-----	15:01,15:11 = 15:02,15:07
++++++-	+-----	-----	-----	15:05,15:19 = 15:06,15:31 =
				15:19,15:36
++++++-	-----+	-----	-----	15:05,15:08 = 15:08,15:36 =
				15:16,15:31
++++++-	-----	-----+	-----	15:02,15:25 = 15:25,15:29 =
				15:25,15:30
++++++-	-----	-----+	-----	15:05,15:30 = 15:30,15:36 =
				15:31,15:37
++++++-	-----	-----+	-----	15:01,15:27 = 15:22,15:27 =
				15:22,15:31 = 15:27,15:36
++++++-	-----	-----+	-----	15:05,15:29 = 15:29,15:36
++++++-	-----	-----+	-----	15:05,15:26 = 15:26,15:36 =
				15:31,15:43
++++++-	-----	-----	+-----	15:05,15:39 = 15:31,15:33 =
				15:36,15:39
++++++-	-----	-----	-+-----	15:05,15:38 = 15:36,15:38
++++++-	-----	-----	-----+	15:05,15:44 = 15:36,15:44
++++++-	-----	-----	-----	15:01,15:31 = 15:02,15:05 =
				15:02,15:36 = 15:31,15:36
++++++-	++-----	-----	-----	15:04,15:19 = 15:06,15:15
++++++-	+-----+	-----	-----	15:13,15:19 = 15:17N,15:19
++++++-	+-----+	-----	-----	15:06,15:08 = 15:16,15:19
++++++-	+-----	-----+	-----	15:06,15:30 = 15:19,15:37
++++++-	+-----	-----+	-----	15:06,15:26 = 15:19,15:43
++++++-	+-----	-----	+-----	15:06,15:39 = 15:19,15:33
++++++-	+-----	-----	-----	15:01,15:19 = 15:02,15:06 =
				15:06,15:19
++++++-	-+-----+	-----	-----	15:04,15:08 = 15:15,15:16
++++++-	-+-----	-----+	-----	15:04,15:30 = 15:15,15:37
++++++-	-+-----	-----+	-----	15:04,15:26 = 15:15,15:43
++++++-	-+-----	-----	+-----	15:04,15:39 = 15:15,15:33
++++++-	-+-----	-----	-----	15:01,15:15 = 15:02,15:04
++++++-	-----++	-----	-----	15:08,15:13 = 15:08,15:17N
++++++-	-----+	-----+	-----	15:13,15:30 = 15:17N,15:30
++++++-	-----+	-----+	-----	15:13,15:29 = 15:17N,15:29
++++++-	-----+	-----+	-----	15:13,15:26 = 15:17N,15:26
++++++-	-----+	-----	+-----	15:13,15:39 = 15:17N,15:39
++++++-	-----+	-----	-+-----	15:13,15:38 = 15:17N,15:38
++++++-	-----+	-----	-----+	15:13,15:44 = 15:17N,15:44

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+++++---	-----+--	-----	-----	15:02,15:13 = 15:02,15:17N
+++++---	-----+--	--+-----	-----	15:02,15:21 = 15:21,15:30
+++++---	-----+--	--+-----	-----	15:08,15:37 = 15:16,15:30
+++++---	-----+--	-----+--	-----	15:08,15:43 = 15:16,15:26
+++++---	-----+--	-----+--	+-----	15:08,15:33 = 15:16,15:39
+++++---	-----+--	-----	-----	15:01,15:08 = 15:02,15:16 =
				15:08,15:16
+++++---	-----	--+-----	-----	15:26,15:37 = 15:30,15:43
+++++---	-----	--+-----	+-----	15:30,15:33 = 15:37,15:39
+++++---	-----	--+-----	-----	15:01,15:30 = 15:02,15:37 =
				15:30,15:37
+++++---	-----	-----+--	-----	15:02,15:40 = 15:24,15:26 =
				15:26,15:40
+++++---	-----	-----+--	+-----	15:26,15:33 = 15:39,15:43
+++++---	-----	-----+--	-----	15:01,15:26 = 15:02,15:43 =
				15:26,15:43
+++++---	-----	-----	+-----	15:01,15:39 = 15:02,15:33 =
				15:33,15:39
+++++---	-----	-----+--	-----	15:03,15:24 = 15:03,15:28
+++++---	-----	-----	-----	15:05,15:07 = 15:07,15:36
+++++---	-----	-----	-----	15:07,15:13 = 15:07,15:17N
+++++---	-----	-----	-----	15:05,15:06 = 15:06,15:36
+++++---	-----	-----	-----	15:05,15:12 = 15:12,15:36
+++++---	-----	-----	-----	15:05,15:09 = 15:09,15:36
+++++---	-----	-----	-----	15:05,15:32 = 15:32,15:36
+++++---	-----	-----	-----	15:05,15:13 = 15:13,15:36 =
				15:17N,15:36
+++++---	-----	-----	-----	15:05,15:16 = 15:16,15:36
+++++---	-----	-----	-----	15:05,15:18 = 15:18,15:36
+++++---	-----	-----	-----	15:05,15:20 = 15:20,15:36
+++++---	-----	-----	-----	15:01,15:25 = 15:25,15:36 =
				15:25,15:37
+++++---	-----	-----	-----	15:05,15:37 = 15:36,15:37
+++++---	-----	-----	-----	15:05,15:22 = 15:22,15:36
+++++---	-----	-----	-----	15:01,15:23 = 15:23,15:36
+++++---	-----	-----	-----	15:05,15:40 = 15:36,15:40
+++++---	-----	-----	-----	15:05,15:24 = 15:24,15:36 =
				15:28,15:36
+++++---	-----	-----	-----	15:05,15:43 = 15:36,15:43
+++++---	-----	-----	-----	15:05,15:33 = 15:33,15:36
+++++---	-----	-----	-----	15:05,15:42 = 15:36,15:42
+++++---	-----	-----	-----	15:05,15:35 = 15:35,15:36
+++++---	-----	-----	-----	15:05,15:41 = 15:36,15:41
+++++---	-----	-----	-----	15:01,15:05 = 15:01,15:36 =
				15:05,15:36 = 15:36,15:36
+++++---	-----	-----	-----	15:06,15:13 = 15:06,15:17N
+++++---	-----	-----	-----	15:06,15:24 = 15:06,15:28
+++++---	-----	-----	-----	15:01,15:06 = 15:06,15:06
+++++---	-----	-----	-----	15:12,15:13 = 15:12,15:17N
+++++---	-----	-----	-----	15:12,15:24 = 15:12,15:28
+++++---	-----	-----	-----	15:01,15:12 = 15:12,15:12
+++++---	-----	-----	-----	15:09,15:13 = 15:09,15:17N
+++++---	-----	-----	-----	15:09,15:24 = 15:09,15:28
+++++---	-----	-----	-----	15:01,15:09 = 15:09,15:09
+++++---	-----	-----	-----	15:13,15:32 = 15:17N,15:32
+++++---	-----	-----	-----	15:10,15:37 = 15:21,15:32
+++++---	-----	-----	-----	15:01,15:10 = 15:10,15:32
+++++---	-----	-----	-----	15:24,15:32 = 15:28,15:32
+++++---	-----	-----	-----	15:01,15:32 = 15:32,15:32
+++++---	-----	-----	-----	15:13,15:16 = 15:16,15:17N
+++++---	-----	-----	-----	15:13,15:18 = 15:17N,15:18
+++++---	-----	-----	-----	15:13,15:20 = 15:17N,15:20

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++++-----	-----+--	--+-----	-----	15:13,15:37 = 15:17N,15:37
++++-----	-----+--	---+-----	-----	15:13,15:22 = 15:17N,15:22
++++-----	-----+--	-----++	-----	15:13,15:40 = 15:17N,15:40
++++-----	-----+--	-----+-	-----	15:13,15:24 = 15:13,15:28 =
				15:17N,15:24
++++-----	-----+--	-----+	-----	15:13,15:43 = 15:17N,15:43
++++-----	-----+--	-----	+-----	15:13,15:33 = 15:17N,15:33
++++-----	-----+--	-----	--+---	15:13,15:42 = 15:17N,15:42
++++-----	-----+--	-----	---+-	15:13,15:35 = 15:17N,15:35
++++-----	-----+--	-----	-----+	15:13,15:41 = 15:17N,15:41
++++-----	-----+--	-----	-----	15:01,15:13 = 15:01,15:17N =
				15:13,15:13 = 15:13,15:17N
++++-----	-----+	--+-----	-----	15:01,15:21 = 15:21,15:37
++++-----	-----+	-----+-	-----	15:16,15:24 = 15:16,15:28
++++-----	-----+	-----	-----	15:01,15:16 = 15:16,15:16
++++-----	-----	+-----	-----	15:18,15:24 = 15:18,15:28
++++-----	-----	+-----	-----	15:01,15:18 = 15:18,15:18
++++-----	-----	+-----	-----	15:20,15:24 = 15:20,15:28
++++-----	-----	+-----	-----	15:01,15:20 = 15:20,15:20
++++-----	-----	--+-----	-----	15:24,15:37 = 15:28,15:37
++++-----	-----	--+-----	-----	15:01,15:37 = 15:37,15:37
++++-----	-----	---++-	-----	15:22,15:24 = 15:22,15:28
++++-----	-----	---+-	-----	15:01,15:22 = 15:22,15:22
++++-----	-----	-----++	-----	15:01,15:40 = 15:24,15:40 =
				15:24,15:43 = 15:28,15:40 =
				15:28,15:43 = 15:40,15:40 =
				15:40,15:43
++++-----	-----	-----+-	+-----	15:24,15:33 = 15:28,15:33
++++-----	-----	-----+-	--+---	15:24,15:42 = 15:28,15:42
++++-----	-----	-----+-	---+-	15:24,15:35 = 15:28,15:35
++++-----	-----	-----+-	-----+	15:24,15:41 = 15:28,15:41
++++-----	-----	-----+-	-----	15:01,15:24 = 15:01,15:28 =
				15:24,15:24 = 15:24,15:28
++++-----	-----	-----+	-----	15:01,15:43 = 15:43,15:43
++++-----	-----	-----	+-----	15:01,15:33 = 15:33,15:33
++++-----	-----	-----	--+---	15:01,15:42 = 15:42,15:42
++++-----	-----	-----	---+-	15:01,15:35 = 15:35,15:35
++++-----	-----	-----	-----+	15:01,15:41 = 15:41,15:41
+++-+-	-----	-----	-----	15:02,15:14 = 15:14,15:14
+++-++	-----	---+-----	-----	15:02,15:34 = 15:11,15:27
+++-+-	+-----	-----	-----	15:02,15:19 = 15:19,15:19
+++-+-	-----+	-----	-----	15:02,15:08 = 15:08,15:08
+++-+-	-----	--+-----	-----	15:02,15:30 = 15:30,15:30
+++-+-	-----	-----+	-----	15:02,15:29 = 15:29,15:29
+++-+-	-----	-----+	-----	15:02,15:26 = 15:26,15:26
+++-+-	-----	-----	+-----	15:02,15:39 = 15:39,15:39
+++-+-	-----	-----	+-----	15:02,15:38 = 15:38,15:38
+++-+-	-----	-----	-----+	15:02,15:44 = 15:44,15:44
+++-++	-----	---+-----	-----	15:03,15:05 = 15:03,15:23
+-++-+	+-----	-----	-----	15:04,15:31 = 15:05,15:15
+-++-+	-----	--+-----	-----	15:05,15:25 = 15:25,15:25
+-++-+	-----	---+-----	-----	15:05,15:23 = 15:23,15:23
+-++-+	-----	---+-----	-----	15:27,15:34 = 15:31,15:34
+-++-+	-----	---+-----	-----	15:27,15:27 = 15:27,15:31

15:01 = 15:01:01:01-15:01:12
15:02 = 15:02:01-15:02:07
15:03 = 15:03:01:01-15:03:01:02

SPECIFICITY TABLE

DRB1*15 SSP subtyping

Specificities and sizes of the PCR products of the 30 primer mixes used for DRB1*15 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB1*15 alleles ³	Other amplified DRB1 alleles ⁴
1	140 bp	515 bp	*15:01:01:01-15:06, 15:08-15:10, 15:12-15:27, 15:29-15:33, 15:35-15:44	*16:09-16:10
2	200 bp	515 bp	*15:01:01:01-15:03:01:02, 15:06-15:09, 15:11-15:14, 15:16, 15:18 ^w , 15:19-15:20, 15:22, 15:24, 15:26, 15:29-15:30, 15:32-15:33, 15:35-15:44	
3	150 bp	430 bp	*15:01:01:01-15:02:07, 15:06-15:09, 15:11-15:14, 15:16, 15:18 ^w , 15:19-15:20, 15:22, 15:24, 15:26, 15:28-15:30, 15:32-15:33, 15:35-15:44	*04:73, 11:82, 13:09, 14:37
4	260 bp	430 bp	*15:01:01:01-15:01:12, 15:03:01:01-15:07, 15:09-15:10, 15:12-15:13, 15:16-15:18, 15:20-15:25, 15:32-15:33, 15:35-15:37, 15:40-15:43	*16:15
5	260 bp	430 bp	*15:02:01-15:02:07, 15:08, 15:11, 15:14-15:15, 15:19, 15:26-15:27, 15:29-15:31, 15:34, 15:38-15:39, 15:44	*16:01:01-16:05:02, 16:07-16:11, 16:13N-16:14, 16:16
6⁷	150 bp, 180 bp	430 bp	*15:03:01:01-15:03:01:02, 15:14	
7	140 bp	430 bp	*15:07, 15:11, 15:34	*16:01:01-16:05:02,

				16:07-16:08, 16:11, 16:13N-16:16
8⁸	200 bp, 245 bp	430 bp	*15:05, 15:23, 15:25, 15:27, 15:31, 15:34, 15:36	
9^{5,9}	90 bp, 225 bp	430 bp	*15:06, 15:19	
10	200 bp	430 bp	*15:04, 15:15	
11	170 bp	430 bp	*15:12	
12⁵	95 bp	430 bp	*15:09	*11:88
13^{5,10}	110 bp, 215 bp	430 bp	*15:10, 15:32	
14	200 bp	430 bp	*15:13, 15:17N	
15	200 bp	515 bp	*15:10, 15:21	*16:05:01-16:05:02, 16:07
16¹¹	135 bp, 200 bp	430 bp	*15:08, 15:16	
17	160 bp	430 bp	*15:18	
18	250 bp	430 bp	*15:20	
19¹²	175 bp, 225 bp	430 bp	*15:21, 15:25, 15:30, 15:37	*16:04
20¹³	165 bp, 200 bp	430 bp	*15:22, 15:27, 15:34	
21	165 bp	430 bp	*15:03:01:01- 15:03:01:02, 15:23	
22^{6,14}	165 bp, 200 bp	430 bp	*15:24, 15:28, 15:40	
23¹⁵	170 bp, 220 bp	430 bp	*15:25, 15:29	
24	200 bp	430 bp	*15:26, 15:40, 15:43	
25^{5,16}	70 bp, 195 bp	430 bp	*15:33, 15:39	
26	180 bp	430 bp	*15:38	
27	170 bp	430 bp	*15:42	
28	255 bp	430 bp	*15:35	
29	190 bp	430 bp	*15:41	
30	220 bp	430 bp	*15:44	

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 DRB*15 SSP subtypings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective lengths of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB1*15 subtyping.

In addition, wells number 2 and 15 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

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

³For several DRB alleles only partial second exon nucleotide sequences are available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. We assume that unknown sequences in the first hyperpolymorphic region of the second exon of DRB alleles are conserved within allelic groups and that unknown sequences of codons 87 to 92 are identical with the DRB1*0101 consensus sequence.

⁴Due to the sharing of sequence motifs between DRB1 alleles, the DRB1* 04:73, 11:82, 13:09 and 14:37 alleles will be amplified by primer mix 3, the DRB1*11:88 allele will be amplified by primer mix 12, and DRB1*16 alleles will be amplified by primer mixes 1, 4, 5, 7, 15 and 19.

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

⁶Primer mix 22 may have a tendency of giving rise to nonspecific amplifications.

⁷Primer mix 6: Specific PCR fragment of 150 bp in the DRB1*15:03:01:01 and 15:03:01:02 alleles. Specific PCR fragment of 180 bp in the DRB1*15:14 allele.

⁸Primer mix 8: Specific PCR fragment of 200 bp in the DRB1*15:05, 15:23, 15:25, 15:27, 15:31 and 15:34 alleles. Specific PCR fragment of 245 bp in the DRB1*15:36 allele.

⁹Primer mix 9: Specific PCR fragment of 90 bp in the DRB1*15:06 allele. Specific PCR fragment of 225 bp in the DRB1*15:19 allele.

¹⁰Primer mix 13: Specific PCR fragment of 110 bp in the DRB1*15:32 allele. Specific PCR fragment of 215 bp in the DRB1*15:10 allele.

¹¹Primer mix 16: Specific PCR fragment of 135 bp in the DRB1*15:16 allele. Specific PCR fragment of 200 bp in the DRB1*15:08 allele.

¹²Primer mix 19: Specific PCR fragment of 175 bp in the DRB1*15:30 allele. Specific PCR fragment of 225 bp in the DRB1*15:21, 15:25 and 15:37 and the DRB1*16:04 alleles.

¹³Primer mix 20: Specific PCR fragment of 165 bp in the DRB1*15:22 allele. Specific PCR fragment of 200 bp in the DRB1*15:27 and 15:34 alleles.

¹⁴Primer mix 22: Specific PCR fragment of 165 bp in the DRB1*15:24 allele. Specific PCR fragment of 200 bp in the DRB1*15:28 and 15:40 alleles.

¹⁵Primer mix 23: Specific PCR fragment of 170 bp in the DRB1*15:29 allele. Specific PCR fragment of 220 bp in the DRB1*15:25 allele.

¹⁶Primer mix 25: Specific PCR fragment of 70 bp in the DRB1*15:39 allele. Specific PCR fragment of 195 bp in the DRB1*15:33 allele.

‘w’, may be weakly amplified.

INTERPRETATION TABLE																
DRB1*15 SSP subtyping																
Amplification patterns of the DRB1*15:01 to 15:44 alleles																
	Well ⁴															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length of spec.	140	200	150	260	260	150	140	200	90	200	170	95	110	200	200	135
PCR product						180		245	225				215			200
Length of int.	515	515	430	430	430	430	430	430	430	430	430	430	430	430	515	430
pos. control ¹																
5'primer(s) ²	13(126)	13(126)	30(175)	13(126)	13(126)	20(146)	13(126)	13(126)	6(103)	13(126)	13(126)	30(175)	13(126)	13(126)	13(126)	13(126)
	5' -Agg 3'	5' -Agg 3'	5' -gAT 3'	5' -Agg 3'	5' -Agg 3'	5' -TgC 3'	5' -Agg 3'	5' -Agg 3'	5' -Cag 3'	5' -Agg 3'	5' -Agg 3'	5' -gAT 3'	5' -Agg 3'	5' -Agg 3'	5' -Agg 3'	5' -Agg 3'
						30(175)			50(236)							
						5' -gAC 3'			5' -ggC 3'							
3'primer(s) ³	47(227)	67(286)	67(286)	86(344)	86(344)	67(286)	47(227)	67(286)	67(286)	67(286)	57(256)	48(230)	37(196)	64(277)	67(286)	45(220)
	5' -ggA 3'	5' -gAT 3'	5' -gAT 3'	5' -CCA 3'	5' -CAC 3'	5' -gAT 3'	5' -ggT 3'	5' -gAg 3'	5' -gAT 3'	5' -gAA 3'	5' -gCT 3'	5' -CCT 3'	5' -ggT 3'	5' -Tg 3'	5' -gAT 3'	5' -CCT 3'
								67(286)					71(299)	69(294)	67(286)	66(283)
								5' -gAg 3'					5' -gCT 3'	5' -TgT 3'	5' -gAT 3'	5' -gTT 3'
								81(329)								
							5' -TgC 3'									
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
DRB1 allele																

INTERPRETATION TABLE													
DRB1*15 SSP subtyping													
Amplification patterns of the DRB1*15:01 to 15:44 alleles													
Well ⁴													
17	18	19	20	21	22	23	24	25	26	27	28	29	30
160	250	175	165	165	165	170	200	70	180	170	255	190	220
		225	200		200	220		195					
430	430	430	430	430	430	430	430	430	430	430	430	430	430
30(175)	13(126)	13(126)	13(126)	30(175)	13(126)	13(126)	15(131)	15(130)	13(126)	23(155)	13(126)	13(126)	13(126)
5' -gAT 3'	5' -Agg 3'	5' -Agg 3'	5' -Agg 3'	5' -gAC 3'	5' -AAG 3'	5' -Agg 3'	5' -gTA 3'	5' -AgA 3'	5' -Agg 3'	5' -gCA 3'	5' -Agg 3'	5' -Agg 3'	5' -Agg 3'
					15(131)		19(143)	58(259)					
					5' -gTA 3'		5' -CAg 3'	5' -ACA 3'					
					25(161)		21(148)						
					5' -gCA 3'		5' -ggT 3'						
70(295)	84(337)	57(258)	55(251)	71(298)	67(286)	57(257)	71(298)	67(286)	60(266)	67(286)	85(341)	62(273)	73(305)
5' -CTC 3'	5' -CCT 3'	5' -gCC 3'	5' -gCA 3'	5' -CgC 3'	5' -gAT 3'	5' -CgA 3'	5' -CgC 3'	5' -gAT 3'	5' -Agg 3'	5' -gAT 3'	5' -CAg 3'	5' -CTC 3'	5' -CgA 3'
		74(307)	67(286)			73(305)							
		5' -CAg 3'	5' -gAg 3'			5' -ggC 3'							
		77(317)											
		5' -AgT 3'											
17	18	19	20	21	22	23	24	25	26	27	28	29	30

Lot No.: **09K**

Lot-specific information

www.olerup-ssp.com

Length of spec.	140	200	150	260	260	150	140	200	90	200	170	95	110	200	200	135
PCR product						180		245	225				215			200
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
DRB1 allele																
*15:01:01-15:01:12	1	2	3	4												
*15:02:01-15:02:07	1	2	3		5											
*15:03:01:01-15:03:01:02	1	2		4		6										
*15:04	1			4						10						
*15:05	1			4				8								
*15:06	1	2	3	4					9							
*15:07		2	3	4			7									
*15:08	1	2	3		5											16
*15:09	1	2	3	4								12				
*15:10	1			4									13		15	
*15:11		2	3		5		7									
*15:12	1	2	3	4							11					
*15:13	1	2	3	4										14		
*15:14	1	2	3		5	6										
*15:15	1				5					10						
*15:16	1	2	3	4												16
*15:17N	1			4										14		
*15:18	1	w	w	4												
*15:19	1	2	3		5				9							
*15:20	1	2	3	4												
*15:21	1			4											15	
*15:22	1	2	3	4												
*15:23	1			4				8								
*15:24	1	2	3	4												
*15:25	1			4				8								
*15:26	1	2	3		5											
*15:27	1				5			8								
*15:28			3													
*15:29	1	2	3		5											
*15:30	1	2	3		5											
*15:31	1				5			8								
*15:32	1	2	3	4									13			
*15:33	1	2	3	4												
*15:34					5		7	8								
*15:35	1	2	3	4												
*15:36	1	2	3	4				8								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Lot No.: **09K**

Lot-specific information

www.olerup-ssp.com

160	250	175	165	165	165	170	200	70	180	170	255	190	220	Length of spec. PCR product
		225	200		200	220		195						
17	18	19	20	21	22	23	24	25	26	27	28	29	30	Well No.
														DRB1 allele
														*15:01:01:01- 15:01:12
														*15:02:01-15:02:07
				21										*15:03:01:01- 15:03:01:02
														*15:04
														*15:05
														*15:06
														*15:07
														*15:08
														*15:09
														*15:10
														*15:11
														*15:12
														*15:13
														*15:14
														*15:15
														*15:16
														*15:17N
17														*15:18
														*15:19
	18													*15:20
		19												*15:21
			20											*15:22
				21										*15:23
					22									*15:24
		19				23								*15:25
							24							*15:26
			20											*15:27
					22									*15:28
						23								*15:29
		19												*15:30
														*15:31
														*15:32
								25						*15:33
			20											*15:34
											28			*15:35
														*15:36
17	18	19	20	21	22	23	24	25	26	27	28	29	30	Well No.

Lot No.: **09K**

Lot-specific information

www.olerup-ssp.com

Length of spec.	140	200	150	260	260	150	140	200	90	200	170	95	110	200	200	135
PCR product						180		245	225				215			200
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*15:37	1	2	3	4												
*15:38	1	2	3		5											
*15:39	1	2	3		5											
*15:40	1	2	3	4												
*15:41	1	2	3	4												
*15:42	1	2	3	4												
*15:43	1	2	3	4												
*15:44	1	2	3		5											
*04:73, 11:82, 13:09, 14:37			3													
*11:88												12				
*16:01:01-16:03, 16:08, 16:11, 16:13N- 16:14, 16:16					5		7									
*16:04					5		7									
*16:05:01-16:05:02, 16:07					5		7								15	
*16:09-16:10	1				5											
*16:15				4			7									
DRB1 allele																
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 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB1*15 subtyping.

In addition, wells number 2 and 15 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

²The codon, and in parenthesis the nucleotide, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given. Codon and 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 codon, and in parenthesis the nucleotide, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Codon and 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.

160	250	175	165	165	165	170	200	70	180	170	255	190	220	Length of spec. PCR product
		225	200		200	220		195						
17	18	19	20	21	22	23	24	25	26	27	28	29	30	Well No.
		19												*15:37
									26					*15:38
								25						*15:39
					22		24							*15:40
												29		*15:41
										27				*15:42
							24							*15:43
												30		*15:44
														*04:73, 11:82, 13:09, 14:37
														*11:88
														*16:01:01-16:03, 16:08, 16:11, 16:13N- 16:14, 16:16
		19												*16:04
														*16:05:01-16:05:02, 16:07
														*16:09-16:10
														*16:15
														DRB1 allele
17	18	19	20	21	22	23	24	25	26	27	28	29	30	Well No.

⁴Primer mix 6: Specific PCR fragment of 150 bp in the DRB1*15:03:01:01 and 15:03:01:02 alleles.

Specific PCR fragment of 180 bp in the DRB1*15:14 allele.

Primer mix 8: Specific PCR fragment of 200 bp in the DRB1*15:05, 15:23, 15:25, 15:27, 15:31 and 15:34 alleles. Specific PCR fragment of 245 bp in the DRB1*15:36 allele.

Primer mix 9: Specific PCR fragment of 90 bp in the DRB1*15:06 allele. Specific PCR fragment of 225 bp in the DRB1*15:19 allele.

Primer mix 13: Specific PCR fragment of 110 bp in the DRB1*15:32 allele. Specific PCR fragment of 215 bp in the DRB1*15:10 allele.

Primer mix 16: Specific PCR fragment of 135 bp in the DRB1*15:16 allele. Specific PCR fragment of 200 bp in the DRB1*15:08 allele.

Primer mix 19: Specific PCR fragment of 175 bp in the DRB1*15:30 allele. Specific PCR fragment of 225 bp in the DRB1*15:21, 15:25 and 15:37 and the DRB1*16:04 alleles.

Primer mix 20: Specific PCR fragment of 165 bp in the DRB1*15:22 allele. Specific PCR fragment of 200 bp in the DRB1*15:27 and 15:34 alleles.

Primer mix 22: Specific PCR fragment of 165 bp in the DRB1*15:24 allele. Specific PCR fragment of 200 bp in the DRB1*15:28 and 15:40 alleles.

Primer mix 23: Specific PCR fragment of 170 bp in the DRB1*15:29 allele. Specific PCR fragment of 220 bp in the DRB1*15:25 allele.

Primer mix 25: Specific PCR fragment of 70 bp in the DRB1*15:39 allele. Specific PCR fragment of 195 bp in the DRB1*15:33 allele.

‘w’, may be weakly amplified.

CELL LINE VALIDATION SHEET																													
DRB1*15 SSP subtyping kit																													
					Well																								
					17	18	19	20	21	22	23	24	25	26	27	28	29	30											
				Prod. No.:	200730117	200956018	201071019	200956020	200844021	201071022	201071023	201071024	201071025	201071026	201071027	201071028	201071029	201071030											
	IHC cell line		DRB1																										
1	9001	SA	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
2	9280	LK707	*15:02	*04:05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
3	9011	E4181324	*15:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
4	9275	GU373	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
5	9009	KAS011	*16:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
6	9353	SM	*04:07	*08:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
7	9020	QBL	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
8	9025	DEU	*04:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
9	9026	YAR	*04:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
10	9107	LKT3	*04:05		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
11	9051	PITOUT	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
12	9052	DBB	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
13	9004	JESTHOM	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
14	9071	OLGA	*08:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
15	9075	DKB	*09:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
16	9037	SWEIG007	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
17	9282	CTM3953540	*03:01	*13:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
18	9257	32367	*09:01	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
19	9038	BM16	*12:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
20	9059	SLE005	*13:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
21	9064	AMALA	*14:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
22	9056	KOSE	*13:02	*14:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
23	9124	IHL	*08:03	*14:14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
24	9035	JBUSH	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
25	9049	IBW9	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
26	9285	WT49	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
27	9191	CH1007	*04:05	*10:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
28	9320	BEL5GB	*04:16	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
29	9050	MOU	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
30	9021	RSH	*03:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
31	9019	DUCAF	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
32	9297	HAG	*13:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
33	9098	MT14B	*04:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
34	9104	DHIF	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
35	9302	SSTO	*04:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
36	9024	KT17	*04:03	*04:06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
37	9065	HHKB	*13:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
38	9099	LZL	*14:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
39	9315	CML	*03:01	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
40	9134	WHONP199	*07:01	*09:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
41	9055	H0301	*13:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
42	9066	TAB089	*08:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
43	9076	T7526	*09:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
44	9057	TEM	*14:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
45	9239	SHJO	*07:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
46	9013	SCHU	*15:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
47	9045	TUBO	*11:04	*12:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										
48	9303	TER-ND	*01:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-										

CERTIFICATE OF ANALYSIS**Olerup SSP® DRB1*15 SSP****Product number:** 101.125-24/06 – including *Taq* pol.**Lot number:** 09K**Expiry date:** 2012-April-01**Number of tests:** 24 test – Product No. 101.125-24

6 tests – Product No. 101.125-06

Number of wells per test: 30**Well specifications:**

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2007-301-01	11	2007-301-11	21	2008-440-21
2	2010-710-02	12	2007-301-12	22	2010-710-22
3	2010-710-03	13	2010-710-13	23	2010-710-23
4	2007-301-04	14	2007-301-14	24	2010-710-24
5	2007-301-05	15	2010-710-15	25	2010-710-25
6	2007-301-06	16	2007-301-16	26	2010-710-26
7	2007-301-07	17	2007-301-17	27	2010-710-27
8	2010-710-08	18	2009-560-18	28	2010-710-28
9	2007-301-09	19	2010-710-19	29	2010-710-29
10	2007-301-10	20	2009-560-20	30	2010-710-30

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 8, 9, 11 to 20 and 22 to 30 were available. The specificities of the primers in primer solutions 8, 11, 13, 15, 19, 20, 22, 23, 26 and 28 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 12, 14, 16 to 18, 29 and 30 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. In primer solutions 9, 24, 25 and 27 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 6 and 22 one respective two of the 5'-primers were not possible to test, and in primer solutions 2, 3, 8, 13, 19, 20 and 23 one or two of the 3'-primers were not possible to test.

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

Date of approval: 2010-May-07

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*15
Product number: 101.125-24/06
Lot number: 09K

Intended use: DRB1*15 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Hasselstigen 1
SE-133 33 Saltsjöbaden, 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, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

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

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom. (Notified Body number: 0088.)

Saltsjöbaden, Sweden
2010-May-07

Olle Olerup
Managing Director

Lot No.: **09K**

Lot-specific information

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