

Olerup SSP® DRB1*01

Product number:	101.111-24u/06u – without <i>Taq</i> pol.
Lot number:	28M
Expiry date:	2013-November-01
Number of tests:	24 test – Product No. 101.111-24u 6 tests – Product No. 101.111-06u
Number of wells per test:	22
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. 28M.

**CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*®
DRB1*01 Lot**

The DRB1*01 specificity and interpretation tables have been updated for the DRB1 alleles described since the previous *Olerup SSP*® DRB1*01 lot was made (Lot No. 90G).

Three wells have been added to the DRB1*01 kit,
wells **20 to 22**.

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

Well	5'-primer	3'-primer	rationale
20	New	New	New primer pair for the DRB1*01:33N allele.
21	New	New	New primer pairs for the DRB1*01:32 and DRB1*01:34 alleles.
22	New	New	New primer pair for the DRB1*01:36 allele.

PRODUCT DESCRIPTION

DRB1*01 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*01:01 to DRB1*01:36 alleles.

PLATE LAYOUT

Each test consists of 22 PCR reactions in a 24 well PCR plate. Wells 23 and 24 are empty.

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

The 24 well PCR plate is marked with 'DRB1*01' in silver/gray ink.

Well No. 1 is marked with the Lot No. '28M'.

A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.

The PCR plates are covered with a PCR-compatible foil.

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

INTERPRETATION

Only the DRB1*01 alleles will be amplified by the DRB1*01 subtyping kit, except that the DRB1*14:112 will be amplified by primer mix 8. Thus, the interpretation of DRB1*01 subtypings is only marginally influenced by other groups of DRB1 alleles.

UNIQUELY IDENTIFIED ALLELES

All the DRB1*01 alleles, i.e. **DRB1*01:01 to DRB1*01:36**, recognized by the HLA Nomenclature Committee in April 2011¹ will give rise to unique amplification patterns by the primers in the DRB1*01 subtyping kit.

The DRB1*01 subtyping kit cannot distinguish the DRB1*01:01:01-01:01:20 alleles or the DRB1*01:02:01-01:02:05 alleles.

¹DRB1 alleles listed on the IMGT/HLA web page 2011-April-08, release 3.4.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

A total of 59 alleles generate 36 amplification patterns that can be combined in 666 homozygous and heterozygous combinations. 224 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.

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Lot-specific information

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+++--+-	---+----	-----	*01:02:01, *01:31 = *01:05, *01:26 = *01:26, *01:31
+++-----	---+----	-----+	*01:12, *01:34 = *01:26, *01:32
+++-----	---+----	-----	*01:01:01, *01:26 = *01:02:01, *01:12 = *01:12, *01:26
+++-----	-----++-	-----	*01:16, *01:23 = *01:23, *01:28
+++-----	---+--+-	-----	*01:01:01, *01:23 = *01:13, *01:23 = *01:14, *01:23
+++-----	-----	-----+	*01:01:01, *01:34 = *01:02:01, *01:32 = *01:32, *01:34
+-----+	-----	-----	*01:03, *01:11 = *01:03, *01:35
+-----	---+-----	-----	*01:10, *01:11 = *01:10, *01:35
+-----+	+-----	-----	*01:03, *01:08 = *01:03, *01:20
+-----+	---+-----	-----	*01:03, *01:10 = *01:03, *01:18 = *01:10, *01:17
+-----+	-----	-----	*01:01:01, *01:03 = *01:03, *01:17
+-----	+-----	-----	*01:08, *01:10 = *01:10, *01:20
+-----	---+-----	-----	*01:01:01, *01:10 = *01:10, *01:10 = *01:10, *01:18
+-----+	+-----	-----	*01:04, *01:05 = *01:04, *01:29 = *01:08, *01:29 = *01:20,
			*01:29
+-----	---+-----	-----	*01:11, *01:31 = *01:12, *01:29 = *01:29, *01:31 = *01:31,
			*01:35
+-----	-----	-----	*01:01:01, *01:29 = *01:05, *01:11 = *01:05, *01:29 = *01:05,
			*01:35 = *01:11, *01:29 = *01:29, *01:29 = *01:29, *01:35
+-----	---+-----	-----	*01:11, *01:15 = *01:15, *01:35
+-----	-----	-----	*01:11, *01:17 = *01:17, *01:35
+-----+	-----	+-----	*01:11, *01:27 = *01:27, *01:35
+-----+	-----	-----	*01:07, *01:11 = *01:07, *01:35
+-----	++-----	-----	*01:04, *01:09 = *01:06, *01:11
+-----	+-----	-----	*01:01:01, *01:04 = *01:04, *01:08 = *01:04, *01:11 = *01:08,
			*01:11 = *01:08, *01:35 = *01:11, *01:20
+-----	---+-----	-----	*01:09, *01:11 = *01:09, *01:35
+-----	---+-----	-----	*01:11, *01:18 = *01:18, *01:35
+-----	---+-----	-----	*01:11, *01:12 = *01:12, *01:35
+-----	---+--+-	-----	*01:11, *01:28 = *01:28, *01:35
+-----	---+--+-	-----	*01:11, *01:13 = *01:13, *01:35
+-----	-----++	-----	*01:11, *01:24 = *01:24, *01:35
+-----	-----+	-----	*01:11, *01:14 = *01:14, *01:35
+-----	-----++	-----	*01:11, *01:21 = *01:21, *01:35
+-----	-----+	-----	*01:11, *01:16 = *01:16, *01:35
+-----	-----+	-----	*01:11, *01:19 = *01:19, *01:35
+-----	-----	+-----	*01:11, *01:22 = *01:22, *01:35
+-----	-----	---+-----	*01:11, *01:25 = *01:25, *01:35
+-----	-----	---+-----	*01:11, *01:30 = *01:30, *01:35
+-----	-----	-----+	*01:11, *01:32 = *01:32, *01:35
+-----	-----	-----+	*01:11, *01:36 = *01:35, *01:36
+-----	-----	-----	*01:01:01, *01:11 = *01:01:01, *01:35 = *01:11, *01:11 =
			*01:11, *01:35
+-----	---+-----	-----	*01:08, *01:31 = *01:20, *01:31
+-----	+-----	-----	*01:05, *01:08 = *01:05, *01:20
+-----	---+-----	-----	*01:01:01, *01:31 = *01:05, *01:12 = *01:05, *01:31 = *01:12,
			*01:31 = *01:31, *01:31
+-----	-----	-----	*01:01:01, *01:05 = *01:05, *01:05
+-----	++-----	-----	*01:06, *01:15 = *01:06, *01:17 = *01:08, *01:15 = *01:15,
			*01:20
+-----	+-----	-----	*01:08, *01:17 = *01:17, *01:20
+-----	---+-----	-----	*01:01:01, *01:15 = *01:09, *01:15 = *01:09, *01:17 = *01:15,
			*01:17
+-----	-----	-----	*01:01:01, *01:17 = *01:17, *01:17
+-----	+-----	+-----	*01:08, *01:27 = *01:20, *01:27
+-----	+-----	-----	*01:07, *01:08 = *01:07, *01:20
+-----	-----	+-----	*01:01:01, *01:27 = *01:07, *01:22 = *01:07, *01:27 = *01:22,

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Lot-specific information

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+ - + - - - - +	- - - - - - - -	- - - - - - - -	*01:27 = *01:27, *01:27
+ - + - - - - -	+ + - - - - - -	- - - - - - - -	*01:01:01, *01:07 = *01:07, *01:07
+ - + - - - - -	+ - + - - - - -	- - - - - - - -	*01:01:01, *01:06 = *01:06, *01:08 = *01:06, *01:09 = *01:08,
			*01:09 = *01:09, *01:20
+ - + - - - - -	+ - + - - - - -	- - - - - - - -	*01:08, *01:18 = *01:18, *01:20
+ - + - - - - -	+ - + - - - - -	- - - - - - - -	*01:08, *01:12 = *01:12, *01:20
+ - + - - - - -	+ - + - - - - -	- - - - - - - -	*01:08, *01:28 = *01:20, *01:28
+ - + - - - - -	+ - + - - - - -	- - - - - - - -	*01:08, *01:13 = *01:13, *01:20
+ - + - - - - -	+ - + - - - - -	- - - - - - - -	*01:08, *01:24 = *01:20, *01:24
+ - + - - - - -	+ - + - - - - -	- - - - - - - -	*01:08, *01:14 = *01:14, *01:20
+ - + - - - - -	+ - + - - - - -	- - - - - - - -	*01:08, *01:21 = *01:20, *01:21
+ - + - - - - -	+ - + - - - - -	- - - - - - - -	*01:08, *01:16 = *01:16, *01:20
+ - + - - - - -	+ - + - - - - -	- - - - - - - -	*01:08, *01:19 = *01:19, *01:20
+ - + - - - - -	+ - + - - - - -	+ - - - - - - -	*01:08, *01:22 = *01:20, *01:22
+ - + - - - - -	+ - + - - - - -	+ - - - - - - -	*01:08, *01:25 = *01:20, *01:25
+ - + - - - - -	+ - + - - - - -	- - + - - - - -	*01:08, *01:30 = *01:20, *01:30
+ - + - - - - -	+ - + - - - - -	- - - - + - - -	*01:08, *01:32 = *01:20, *01:32
+ - + - - - - -	+ - + - - - - -	- - - - + - - -	*01:08, *01:36 = *01:20, *01:36
+ - + - - - - -	+ - + - - - - -	- - - - - - - -	*01:01:01, *01:08 = *01:01:01, *01:20 = *01:08, *01:08 =
			*01:08, *01:20
+ - + - - - - -	- + - - - - - -	- - - - - - - -	*01:01:01, *01:09 = *01:09, *01:09
+ - + - - - - -	- - + - - - - -	- - - - - - - -	*01:01:01, *01:18 = *01:18, *01:18
+ - + - - - - -	- - - + - - - -	- - - - - - - -	*01:01:01, *01:12 = *01:12, *01:12
+ - + - - - - -	- - - - + - + -	- - - - - - - -	*01:13, *01:21 = *01:19, *01:28 = *01:21, *01:28
+ - + - - - - -	- - - - + - + -	- - - - - - - -	*01:01:01, *01:28 = *01:13, *01:16 = *01:13, *01:28 = *01:16,
			*01:28 = *01:28, *01:28
+ - + - - - - -	- - - - + - - -	- - - - - - - -	*01:01:01, *01:13 = *01:13, *01:13
+ - + - - - - -	- - - - + + + -	- - - - - - - -	*01:14, *01:21 = *01:16, *01:24 = *01:21, *01:24
+ - + - - - - -	- - - - + - + -	- - - - - - - -	*01:01:01, *01:24 = *01:14, *01:19 = *01:14, *01:24 = *01:19,
			*01:24
+ - + - - - - -	- - - - + - - -	- - - - - - - -	*01:01:01, *01:14 = *01:14, *01:14
+ - + - - - - -	- - - - + + + -	- - - - - - - -	*01:01:01, *01:21 = *01:16, *01:19 = *01:16, *01:21 = *01:19,
			*01:21 = *01:21, *01:21
+ - + - - - - -	- - - - + - - -	- - - - - - - -	*01:01:01, *01:16 = *01:16, *01:16
+ - + - - - - -	- - - - - - + -	- - - - - - - -	*01:01:01, *01:19 = *01:19, *01:19
+ - + - - - - -	- - - - - - - -	+ - - - - - - -	*01:01:01, *01:22 = *01:22, *01:22
+ - + - - - - -	- - - - - - - -	- + - - - - - -	*01:01:01, *01:25 = *01:25, *01:25
+ - + - - - - -	- - - - - - - -	- - + - - - - -	*01:01:01, *01:30 = *01:30, *01:30
+ - + - - - - -	- - - - - - - -	- - - - + - - -	*01:01:01, *01:32 = *01:32, *01:32
+ - + - - - - -	- - - - - - - -	- - - - + - - -	*01:01:01, *01:36 = *01:36, *01:36
- + + - - - - -	- - - + - - - -	- - - - - - - -	*01:02:01, *01:26 = *01:26, *01:26
- + + - - - - -	- - - - - - - -	- - - - + - - -	*01:02:01, *01:34 = *01:34, *01:34
- - + - + - - -	+ + - - - - - -	- - - - - - - -	*01:04, *01:06 = *01:06, *01:35
- - + - + - - -	+ - - - - - - -	- - - - - - - -	*01:04, *01:04 = *01:04, *01:20 = *01:04, *01:35 = *01:20,
			*01:35
- - + - - - - -	+ + - - - - - -	- - - - - - - -	*01:06, *01:06 = *01:06, *01:20

*01:01:01 = *01:01:01-01:01:20

*01:02:01 = *01:02:01-01:02:05

SPECIFICITY TABLE**DRB1*01 SSP subtyping**

Specificities and sizes of the PCR products of the 22 primer mixes used for DRB1*01 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB1*01 alleles ³	Other amplified DRB1 alleles ⁴
1⁶	255 bp	515 bp	*01:01:01-01:01:20, 01:03, 01:05, 01:07-01:19, 01:21, 01:22 ^w , 01:24-01:25, 01:27-01:32, 01:36	
2	235 bp	430 bp	*01:02:01-01:02:05, 01:23, 01:26, 01:34	
3	200 bp	515 bp	*01:01:01-01:02:05, 01:04-01:14, 01:16-01:21, 01:22 ^w , 01:25-01:32, 01:34-01:36	
4	210 bp	430 bp	*01:03, 01:10	
5	220 bp	430 bp	*01:04, 01:11, 01:29, 01:35	
6^{7,8}	135 bp, 210 bp	430 bp	*01:05, 01:29, 01:31	
7	210 bp	430 bp	*01:03, 01:15, 01:17	
8⁹	175 bp, 210 bp	430 bp	*01:07, 01:27	*14:112
9^{5,10}	110 bp, 255 bp	430 bp	*01:04, 01:06, 01:08, 01:20	
10	210 bp	430 bp	*01:06, 01:09, 01:15	
11^{6,11}	140 bp, 210 bp	430 bp	*01:10, 01:18	
12¹²	140 bp, 180 bp, 215 bp	430 bp	*01:12, 01:26, 01:31	
13^{5,13}	85 bp, 150 bp, 215 bp	430 bp	*01:13, 01:23, 01:28	
14¹⁴	170 bp, 210 bp	430 bp	*01:14, 01:23-01:24	
15¹⁵	150 bp, 220 bp	430 bp	*01:16, 01:21, 01:28	
16¹⁶	200 bp, 230 bp	430 bp	*01:19, 01:21, 01:24	
17¹⁷	230 bp, 260 bp	430 bp	*01:22, 01:27	
18	230 bp	430 bp	*01:25	

19⁵	125 bp	430 bp	*01:30
20⁶	205 bp	515 bp	*01:33N
21^{5,7,18}	85 bp, 250 bp	430 bp	*01:32, 01:34
22	205 bp	430 bp	*01:36

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*01 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*01 subtyping.

In addition, wells number 3 and 20 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*01:01 consensus sequence.

⁴Due to the sharing of sequence motifs between DRB1 alleles, primer mix 8 will amplify the DRB1*14:112 allele.

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

⁶Primer mixes 1, 11 and 20 have a tendency of giving rise to primer oligomer formation.

⁷Primer mixes 6 and 21 have a tendency of giving rise to non-specific amplification.

⁸Primer mix 6: Specific PCR fragment of 135 bp in the DRB1*01:05 and DRB1*01:31 alleles. Specific PCR fragment of 210 bp in the DRB1*01:29 allele.

⁹Primer mix 8: Specific PCR fragment of 175 bp in the DRB1*01:27 and in the DRB1*14:112 alleles. Specific PCR fragment of 210 bp in the DRB1*01:07 allele.

¹⁰Primer mix 9: Specific PCR fragment of 110 bp in the DRB1*01:08 allele. Specific PCR fragment of 255 bp in the DRB1*01:04, 01:06 and 01:20 alleles.

¹¹Primer mix 11: Specific PCR fragment of 140 bp in the DRB1*01:18 allele. Specific PCR fragment of 210 bp in the DRB1*01:10 allele.

¹²Primer mix 12: Specific PCR fragment of 140 bp in the DRB1*01:31 allele. Specific PCR fragment of 180 bp in the DRB1*01:26 allele. Specific PCR fragment of 215 bp in the DRB1*01:12 allele.

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¹³Primer mix 13: Specific PCR fragment of 85 bp in the DRB1*01:13 allele. Specific PCR fragment of 150 bp in the DRB1*01:28 allele. Specific PCR fragment of 215 bp in the DRB1*01:23 allele.

¹⁴Primer mix 14: Specific PCR fragment of 170 bp in the DRB1*01:14 allele. Specific PCR fragment of 210 bp in the DRB1*01:23 and DRB1*01:24 alleles.

¹⁵Primer mix 15: Specific PCR fragment of 150 bp in the DRB1*01:28 allele. Specific PCR fragment of 220 bp in the DRB1*01:16 and DRB1*01:21 alleles.

¹⁶Primer mix 16: Specific PCR fragment of 200 bp in the DRB1*01:24 allele. Specific PCR fragment of 230 bp in the DRB1*01:19 and DRB1*01:21 alleles.

¹⁷Primer mix 17: Specific PCR fragment of 230 bp in the DRB1*01:27 allele. Specific PCR fragment of 260 bp in the DRB1*01:22 allele.

¹⁸Primer mix 21: Specific PCR fragment of 85 bp in the DRB1*01:34 allele. Specific PCR fragment of 250 bp in the DRB1*01:32 allele.

'w', might be weakly amplified.

INTERPRETATION TABLE**DRB1*01 SSP subtyping**

Amplification patterns of the DRB1*01:01 to 01:36 alleles

	Well ⁴											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	255	235	200	210	220	135	210	175	110	210	140	140
PCR product(s)						210		210	255		210	180
												215
Length of int.	515	430	515	430	430	430	430	430	430	430	430	430
pos. control ¹												
5'-primer(s) ²	14 (129)	14 (129)	14 (129)	14 (129)	14 (129)	14 (129)	14 (129)	10 (115)	14 (129)	14 (129)	14 (129)	14 (129)
	5'-gAA 3'	5'-gAA 3'	5'-gAA 3'	5'-gAA 3'	5'-gAA 3'	5'-gAA 3'	5'-gAA 3'	5'-ggg 3'	5'-gAA 3'	5'-gAA 3'	5'-gAA 3'	5'-gAA 3'
								23 (154)				
								5'-Agg 3'				
3'-primer(s) ³	86 (344)	78 (321)	67 (286)	71 (299)	70 (296)	45 (220)	67 (286)	67 (286)	37 (197)	71 (298)	47 (227)	48 (230)
	5'-CAC 3'	5'-CAA 3'	5'-gAg 3'	5'-gCT 3'	5'-TCC 3'	5'-CCT 3'	5'-gAT 3'	5'-gAg 3'	5'-CgT 3'	5'-CgC 3'	5'-ggA 3'	5'-CCT 3'
			67 (286)		77 (317)	48 (230)	67 (286)		85 (341)		71 (298)	60 (266)
			5'-gAg 3'		5'-AAT 3'	5'-CCT 3'	5'-gAT 3'		5'-CAA 3'		5'-CTT 3'	5'-Agg 3'
						70 (296)	74 (308)					72 (302)
						5'-TCC 3'	5'-CCT 3'					5'-CCA 3'
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
DRB1 allele												
*01:01:01-01:01:20	1		3									
*01:02:01-01:02:05		2	3									
*01:03	1			4			7					
*01:04			3		5				9			
*01:05	1		3			6						
*01:06			3						9	10		
*01:07	1		3					8				
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE										
DRB1*01 SSP subtyping										
Amplification patterns of the DRB1*01:01 to 01:36 alleles										
Well ⁴										
13	14	15	16	17	18	19	20	21	22	
85	170	150	200	230	230	125	205	85	205	Length of spec.
150	210	220	230	260				250		PCR product(s)
215										
430	430	430	430	430	430	430	515	430	430	Length of int.
										pos. control ¹
14 (129)	14 (129)	14 (129)	14 (129)	12 (123)	23 (154)	59 (262)	13 (124)	14 (129)	14 (129)	5'-primer(s) ²
5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	5'-AAC ^{3'}	5'-AgT ^{3'}	5'-CCA ^{3'}	5'-A.T ^{3'}	5'-gAA ^{3'}	5'-gAA ^{3'}	
				23 (154)						
				5'-Agg ^{3'}						
30 (175)	57 (257)	51 (239)	67 (286)	86 (344)	86 (344)	86 (344)	67 (286)	29 (173)	69 (293)	3'-primer(s) ³
5'-gAg ^{3'}	5'-CAg ^{3'}	5'-CCA ^{3'}	5'-gAT ^{3'}	5'-CAC ^{3'}	5'-CAC ^{3'}	5'-CAC ^{3'}	5'-gAg ^{3'}	5'-ATC ^{3'}	5'-gCC ^{3'}	
51 (239)	67 (286)	73 (305)	76 (313)					84 (337)		
5'-CCA ^{3'}	5'-gAT ^{3'}	5'-ggC ^{3'}	5'-gTT ^{3'}					5'-CCT ^{3'}		
72 (303)	72 (303)	78 (319)	78 (319)							
5'-gCg ^{3'}	5'-gCg ^{3'}	5'-CAC ^{3'}	5'-CAC ^{3'}							
13	14	15	16	17	18	19	20	21	22	Well No.
										DRB1 allele
										*01:01:01-01:01:20
										*01:02:01-01:02:05
										*01:03
										*01:04
										*01:05
										*01:06
										*01:07
13	14	15	16	17	18	19	20	21	22	Well No.

Length of spec.	255	235	200	210	220	135	210	175	110	210	140	140
PCR product(s)						210		210	255		210	180
												215
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*01:08	1		3						9			
*01:09	1		3							10		
*01:10	1		3	4							11	
*01:11	1		3		5							
*01:12	1		3									12
*01:13	1		3									
*01:14	1		3									
*01:15	1						7			10		
*01:16	1		3									
*01:17	1		3				7					
*01:18	1		3								11	
*01:19	1		3									
*01:20			3						9			
*01:21	1		3									
*01:22	w		w									
*01:23		2										
*01:24	1											
*01:25	1		3									
*01:26		2	3									12
*01:27	1		3					8				
*01:28	1		3									
*01:29	1		3		5	6						
*01:30	1		3									
*01:31	1		3			6						12
*01:32	1		3									
*01:33N												
*01:34		2	3									
*01:35			3		5							
*01:36	1		3									
*14:112								8				
DRB1 allele												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **28M**

Lot-specific information

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85	170	150	200	230	230	125	205	85	205	Length of spec.
150	210	220	230	260				250		PCR product(s)
215										
13	14	15	16	17	18	19	20	21	22	Well No.
										*01:08
										*01:09
										*01:10
										*01:11
13										*01:12
	14									*01:13
										*01:14
										*01:15
		15								*01:16
										*01:17
										*01:18
			16							*01:19
		15	16							*01:20
				17						*01:21
13	14									*01:22
	14		16							*01:23
					18					*01:24
										*01:25
				17						*01:26
										*01:27
13		15								*01:28
										*01:29
						19				*01:30
										*01:31
								21		*01:32
							20			*01:33N
								21		*01:34
										*01:35
									22	*01:36
										*14:112
13	14	15	16	17	18	19	20	21	22	DRB1 allele
										Well No.

Lot No.: **28M**

Lot-specific information

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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*01 subtyping.

In addition, wells number 3 and 20 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.

⁴Primer mix 6: Specific PCR fragment of 135 bp in the DRB1*01:05 and DRB1*01:31 alleles. Specific PCR fragment of 210 bp in the DRB1*01:29 allele.

Primer mix 8: Specific PCR fragment of 175 bp in the DRB1*01:27 and in the DRB1*14:112 alleles. Specific PCR fragment of 210 bp in the DRB1*01:07 allele.

Primer mix 9: Specific PCR fragment of 110 bp in the DRB1*01:08 allele. Specific PCR fragment of 255 bp in the DRB1*01:04, 01:06 and 01:20 alleles.

Primer mix 11: Specific PCR fragment of 140 bp in the DRB1*01:18 allele. Specific PCR fragment of 210 bp in the DRB1*01:10 allele.

Primer mix 12: Specific PCR fragment of 140 bp in the DRB1*01:31 allele. Specific PCR fragment of 180 bp in the DRB1*01:26 allele. Specific PCR fragment of 215 bp in the DRB1*01:12 allele.

Primer mix 13: Specific PCR fragment of 85 bp in the DRB1*01:13 allele. Specific PCR fragment of 150 bp in the DRB1*01:28 allele. Specific PCR fragment of 215 bp in the DRB1*01:23 allele.

Primer mix 14: Specific PCR fragment of 170 bp in the DRB1*01:14 allele. Specific PCR fragment of 210 bp in the DRB1*01:23 and DRB1*01:24 alleles.

Primer mix 15: Specific PCR fragment of 150 bp in the DRB1*01:28 allele. Specific PCR fragment of 220 bp in the DRB1*01:16 and DRB1*01:21 alleles.

Primer mix 16: Specific PCR fragment of 200 bp in the DRB1*01:24 allele. Specific PCR fragment of 230 bp in the DRB1*01:19 and DRB1*01:21 alleles.

Primer mix 17: Specific PCR fragment of 230 bp in the DRB1*01:27 allele. Specific PCR fragment of 260 bp in the DRB1*01:22 allele.

Primer mix 21: Specific PCR fragment of 85 bp in the DRB1*01:34 allele. Specific PCR fragment of 250 bp in the DRB1*01:32 allele.

'w', might be weakly amplified.

CELL LINE VALIDATION SHEET											
DRB1*01 SSP subtyping kit											
					Production No.	Well					
						17	18	19	20	21	22
						201070317	201070318	201070319	201186920	201186921	201186922
	IHCW 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	*14:54		-	-	-	-	-	-
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*01 SSP

Product number: 101.111-24u/06u – without *Taq* pol.
Lot number: 28M
Expiry date: 2013-November-01
Number of tests: 24 test – Product No. 101.111-24u
 6 tests – Product No. 101.111-06u
Number of wells per test: 22

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2008-424-01	9	2008-555-09	17	2010-703-17
2	2008-424-02	10	2008-424-10	18	2010-703-18
3	2008-424-03	11	2010-703-11	19	2010-703-19
4	2008-424-04	12	2010-703-12	20	2011-869-20
5	2010-703-05	13	2010-703-13	21	2011-869-21
6	2010-703-06	14	2010-703-14	22	2011-869-22
7	2010-703-07	15	2010-703-15		
8	2010-703-08	16	2010-703-16		

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 5, 6 and 8 to 22 were available. The specificities of the primers in primer solutions 5, 6 and 9 to 16 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 8 and 17 to 20 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solution 21 and 22 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 6 and 12 to 16 one or two 3'-primers were not possible to test. Additional 3'-primers in primer solution 7 were tested by separately adding one additional 5'-primer.

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

Date of approval: 2011-June-10

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup SSP*[®] DRB1*01

Product number: 101.111-24u/06u

Lot number: 28M

Intended use: DRB1*01 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.

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.)

Stockholm, Sweden
2011-June-10

Olle Olerup
Managing Director

Lot No.: **28M**

Lot-specific information

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