

## Olerup SSP® DRB3

Product number:	101.121-24/04 – including <i>Taq</i> pol.
Lot number:	14F
Expiry date:	2010-October-01
Number of tests:	24 tests – Product No. 101.121-24 4 tests – Product No. 101.121-04
Number of wells per test:	24
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

**This Product Description is only valid for Lot No. 14F.**

### CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*® DRB3 LOT

The DRB3 primer set as well as the specificity and interpretation tables have been updated for the DRB alleles described since the previous *Olerup SSP*® DRB3 lot (**Lot No. Y16**) was made.

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

Well	5'-primer	3'-primer	rationale
1	-	Removed, modified	Specific primer for DRB3*0217 moved to well 15 to reduce dimer formation. Modified 3'-primer to increase specific amplification.
9	New	-	Primer added for the DRB3*0212 allele.
15	-	New	Primer added for the DRB3*0217 allele.
16	-	Removed	Specific primer for DRB3*0216 moved to well 17. Change of positive control primer pair to reduce dimer formation.
17	-	Modified, new	Primers added for the DRB3*0216 and 0223 alleles. Modified primer to increase amplification specificity.
19	-	New	Primer added for the DRB3*0223 allele.
21	-	Removed	Specific primer for DRB3*0216 moved to well 16 to reduce dimer formation.

## PRODUCT DESCRIPTION

### DRB3 SSP subtyping

#### CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB3\*0101 to DRB3\*0303 alleles.

#### PLATE LAYOUT

Each test consists of 24 PCR reactions in a 24 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

The 24 well cut PCR plate is marked with 'DRB3' in silver/gray ink.

Well No. 1 is marked with the Lot No. '14F'.

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 alleles of the DRB3 locus will be amplified by the DRB3 subtyping kit, except that primer mixes 1, 2, 5, 8, 12 to 14, 16, 17, 19, 20 and 24 will amplify a few DRB1 alleles (0337, 0338, 0807, 0819, 0825, 0834, 100101 and 100102, 1130, 1143, 1144, 1149, 1150, 1331, 1346, 1354, 1367, 1377, 1446, 1448, 1459). Thus, the interpretation of DRB3 subtypings is only marginally influenced by other DRB genes.

#### UNIQUELY IDENTIFIED ALLELES

All the DRB3 alleles, i.e. **DRB3\*0101 to DRB3\*0111, DRB3\*0201 to DRB3\*0223 and DRB3\*0301 to DRB3\*0303**, recognized by the HLA Nomenclature Committee in July 2008<sup>1</sup> will give rise to unique amplification patterns by the primers in the DRB3 subtyping kit.

The DRB3 subtyping kit cannot distinguish the DRB3\*01010201 to DRB3\*010104 alleles or the DRB3\*020201 to DRB3\*020205 alleles or the DRB3\*030101 and DRB3\*030102 alleles.

<sup>1</sup>DRB alleles listed on the IMGT/HLA web page 2008-July-11, release 2.22.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

**RESOLUTION IN HOMO- AND HETEROZYGOTES**

The 37 phenotypically different DRB3 alleles can be combined in 703 homozygous and heterozygous combinations. 245 of these genotypes do not give rise to unique amplification patterns. (The different sizes of the specific PCR products generated by primer mixes 12, 15, 17 and 19 to 22 were not considered in these calculations.)

++++-----	+-----+--	---+-----	0102,0107 = 0102,0205
++++-----	-----+--	---+-----	0101,0102 = 0102,0102
+++--+-	+-----+--	---+-----	0104,0107 = 0104,0205
+++--+-	-----+--	---+-----	0101,0104 = 0104,0104
+++--+-	+-----+--	---+-----	0105,0107 = 0105,0205
+++--+-	-----+--	---+-----	0101,0105 = 0105,0105
+++-----+	++-----+	+--+-----	0106,0216 = 0108,0216
+++-----+	++-----+	+--+-----	0106,0223 = 0108,0208 = 0108,0223
+++-----+	++-----+	+--+-----	0106,0206 = 0108,0202 = 0108,0206
+++-----+	++-----+	+--+-----	0106,0220 = 0108,0220
+++-----+	+-----+--	+--+-----	0107,0108 = 0108,0205
+++-----+	+-----+--	---+-----	0106,0107 = 0106,0205
+++-----+	-----+--	+--+-----	0101,0108 = 0106,0108 = 0108,0108
+++-----+	-----+--	---+-----	0101,0106 = 0106,0106
+++-----	++-----+	---+-----	0110,0212 = 0111,0211
+++-----	++-----+	---+-----	0101,0211 = 0110,0202 = 0110,0211
+++-----	++-----+	---+-----	0101,0212 = 0111,0202 = 0111,0212
+++-----	+-----+--	---+-----	0107,0110 = 0110,0205
+++-----	+-----+--	---+-----	0107,0111 = 0111,0205
+++-----	+-----+--	---+-----	0101,0107 = 0101,0205
+++-----	-----+--	---+-----	0101,0110 = 0110,0110
+++-----	-----+--	---+-----	0101,0111 = 0111,0111
++-----	+++-----+	---+-----	0219,0301 = 0222,0301
++-----	+++-----+	+--+-----	0206,0301 = 0216,0301
++-----	+++-----+	+--+-----	0207,0301 = 0221,0301
++-----	+++-----+	---+-----	0208,0301 = 0218,0301
++-----	+++-----+	---+-----	0219,0303 = 0222,0303
++-----	+++-----+	+--+-----	0206,0303 = 0216,0303
++-----	+++-----+	+--+-----	0207,0303 = 0221,0303
++-----	+++-----+	---+-----	0208,0303 = 0218,0303
+--+-----	++-----+	+-----	0107,0206 = 0107,0220
+--+-----	++-----+	---+-----	0107,0208 = 0107,0214
+--+-----	+-----+--	-----	0107,0107 = 0107,0205
+-----	++-----+	+-----	0205,0206 = 0205,0220
+-----	++-----+	---+-----	0205,0208 = 0205,0214
-----	++++-----+	+--+-----	0109,0201 = 0109,0204
-----	++++-----+	-----+--	0201,0219 = 0204,0219
-----	++++-----+	-----	0201,0215 = 0201,0222 = 0202,0204 =
-----	++++-----+	-----	0204,0215 = 0204,0222
-----	++++-----+	-----	0201,0204 = 0204,0204
-----	+++-----+	---+-----	0215,0302 = 0219,0302 = 0222,0302
-----	+++-----+	+--+-----	0206,0302 = 0216,0302

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-----	+++-----	---+-----+	0207,0302 = 0221,0302
-----	+++-----	---+-----+	0208,0302 = 0218,0302
-----	++-----	-----	0215,0217 = 0217,0222
-----	++-----	+-----	0206,0217 = 0217,0220
-----	++-----	---+-----	0208,0217 = 0214,0217
-----	++-----	-----	0202,0217 = 0203,0217 = 0217,0217
-----	++-----	+-----	0215,0216 = 0216,0222
-----	++-----	-----	0203,0215 = 0203,0222
-----	++-----	++-----+	0209,0216 = 0216,0221
-----	++-----	+-----	0208,0216 = 0214,0216 = 0216,0223
-----	++-----	+-----	0202,0216 = 0203,0216 = 0206,0216 =
			0210,0216 = 0216,0216 = 0216,0220
-----	++-----	++-----	0109,0206 = 0109,0220
-----	++-----	+-----	0215,0223 = 0222,0223
-----	++-----	+-----	0206,0219 = 0219,0220
-----	++-----	+-----	0206,0215 = 0206,0222 = 0215,0220 =
			0220,0222
-----	++-----	+++-----	0109,0208 = 0109,0214
-----	++-----	---+-----+	0109,0219 = 0109,0221 = 0209,0215 =
			0209,0219 = 0209,0222
-----	++-----	---+-----	0109,0202 = 0109,0207 = 0109,0215 =
			0109,0222
-----	++-----	---+-----	0207,0219 = 0215,0221 = 0219,0221 =
			0221,0222
-----	++-----	---+-----	0207,0215 = 0207,0222
-----	++-----	---+-----+	0208,0219 = 0214,0219 = 0215,0218 =
			0218,0219 = 0218,0222
-----	++-----	---+-----	0208,0215 = 0208,0222 = 0214,0215 =
			0214,0222
-----	++-----	---+-----	0210,0215 = 0210,0222
-----	++-----	-----+-----	0211,0215 = 0211,0222
-----	++-----	-----+-----	0212,0215 = 0212,0222
-----	++-----	-----+-----	0213,0215 = 0213,0222
-----	++-----	-----+-----	0202,0219 = 0215,0219 = 0219,0219 =
			0219,0222
-----	++-----	-----	0202,0215 = 0202,0222 = 0215,0222 =
			0222,0222
-----	++-----	++-----+	0206,0209 = 0209,0220
-----	++-----	++-----+	0206,0221 = 0220,0221
-----	++-----	++-----	0206,0207 = 0207,0220
-----	++-----	+-----+	0206,0218 = 0218,0220 = 0218,0223
-----	++-----	+-----	0202,0223 = 0206,0208 = 0206,0214 =
			0206,0223 = 0208,0220 = 0208,0223 =
			0214,0223 = 0220,0223 = 0223,0223
-----	++-----	+-----	0206,0210 = 0210,0220
-----	++-----	+-----	0206,0211 = 0211,0220
-----	++-----	+-----	0206,0212 = 0212,0220
-----	++-----	+-----	0206,0213 = 0213,0220

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----- ++---+--- +-----	0202,0206 = 0207,0220 = 0206,0206 = 0206,0220
----- ++---+--- -+++-----	0208,0209 = 0209,0214 = 0209,0218
----- ++---+--- -++-----	0207,0218 = 0208,0221 = 0214,0221 = 0218,0221
----- ++---+--- -++-----	0207,0208 = 0207,0214
----- ++---+--- -+-+-----	0202,0209 = 0207,0209 = 0209,0221 = 0210,0221
----- ++---+--- -+-----+	0202,0221 = 0207,0221 = 0221,0221
----- ++---+--- -+-----	0202,0207 = 0207,0207
----- ++---+--- --++-----	0208,0210 = 0210,0214
----- ++---+--- --++-----	0208,0211 = 0211,0214
----- ++---+--- --++-----	0208,0212 = 0212,0214
----- ++---+--- --++-----	0208,0213 = 0213,0214
----- ++---+--- --++-----	0202,0218 = 0208,0218 = 0214,0218 = 0218,0218
----- ++---+--- --++-----	0202,0208 = 0202,0214 = 0208,0208 = 0208,0214
----- ++---+--- -----+---	0202,0211 = 0211,0211
----- ++---+--- -----+---	0202,0212 = 0212,0212
----- ++---+--- -----+---	0202,0213 = 0213,0213
----- -+---+---+ -+-+-----	0109,0109 = 0109,0210
----- -+---+---+ -+-+-----	0209,0209 = 0209,0210

## SPECIFICITY TABLE

### DRB3 SSP subtyping

Specificities and sizes of the PCR products of the 24 primer mixes used for DRB3 SSP subtyping

Primer Mix	Approx. size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified DRB3 alleles <sup>3</sup>	Amplified DRB1 <sup>4</sup> alleles
<b>1<sup>5,6,14</sup></b>	100 bp	<b>515 bp</b>	01010201-0108, 0110, 0111, 0205, 030101-030102, 0303	1130 <sup>weakly</sup> , 1367, 1446
<b>2<sup>5</sup></b>	125 bp	430 bp	01010201-0106, 0108, 0110, 0111, 030101-030102, 0303	0338 <sup>weakly</sup> , 0807 <sup>weakly</sup> , 0819 <sup>weakly</sup> , 0825 <sup>weakly</sup> , 0834 <sup>weakly</sup> , 1331 <sup>weakly</sup> , 1346 <sup>weakly</sup> , 1354 <sup>weakly</sup> , 1377 <sup>weakly</sup> , 1448 <sup>weakly</sup>
<b>3<sup>5</sup></b>	95 bp	<b>515 bp</b>	01010201-0102, 0104-0108, 0110, 0111	
<b>4<sup>5</sup></b>	85 bp	430 bp	0102	
<b>5<sup>5</sup></b>	95 bp	430 bp	0103	1446
<b>6</b>	190 bp	430 bp	0104	
<b>7<sup>5</sup></b>	90 bp	<b>515 bp</b>	0105	
<b>8<sup>5</sup></b>	120 bp	430 bp	0106, 0108	1446
<b>9</b>	165 bp	430 bp	0107, 0201-0208, 0211-0223	
<b>10<sup>7</sup></b>	185 bp	430 bp	0109, 0201-0204, 0206-0214, 0216-0223, 0302	
<b>11</b>	270 bp	<b>515 bp</b>	0201, 0204, 030101- 0302	
<b>12<sup>5,8</sup></b>	90, 145 bp	<b>515 bp</b>	0201, 0204, 0217	0337, 1143, 1150, 1459
<b>13</b>	270 bp	<b>515 bp</b>	01010201- 0111, 020201-0203, 0205-0213, 0215-0223, 0303	1130, 1367, 1446

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<b>14<sup>6</sup></b>	185 bp	<b>515 bp</b>	0107, 0109, 020201-020205, 0205-0213, 0215- 0219, 0221-0223	100101- 100102 <sup>weakly</sup> , 1003 <sup>weakly</sup> , 1149 <sup>weakly</sup>
<b>15<sup>5,7,9</sup></b>	120, 180, 210 bp	430 bp	0203, 0216, 0217, 030101-0303	
<b>16<sup>5,6,7</sup></b>	155 bp	<b>515 bp</b>	0109, 0204, 0215, 0219, 0222	1144 <sup>weakly</sup> , 1149 <sup>weakly</sup> , 1150 <sup>weakly</sup>
<b>17<sup>5,10</sup></b>	120, 180 bp	430 bp	0108, 0206, 0216, 0220, 0223	1367, 1446
<b>18<sup>5</sup></b>	100 bp	430 bp	0109, 0207, 0209, 0221	
<b>19<sup>11</sup></b>	180, 270 bp	<b>515 bp</b>	0208, 0214, 0218, 0223	1130
<b>20<sup>12</sup></b>	165, 180 bp	430 bp	01010201-0106, 0108-0111, 0209, 0210, 0216, 030101-0303	1130, 1367, 1446
<b>21<sup>5,7,13</sup></b>	125, 210 bp	430 bp	0110, 0211	
<b>22<sup>7,14</sup></b>	185, 245 bp	430 bp	0111, 0212	
<b>23<sup>7</sup></b>	195 bp	<b>515 bp</b>	0213	
<b>24</b>	185 bp	430 bp	0209, 0218, 0219, 0221, 030101-0303	1130

<sup>1</sup>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 DRB3 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 length 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.

<sup>2</sup>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 longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB3subtyping.

In addition, wells number 3, 7, 11 to 14, 16, 19 and 23 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.

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<sup>3</sup>For 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.

<sup>4</sup>Due to the sharing of sequence motifs between DRB3 and DRB1 alleles, primer mixes 1, 2, 5, 8, 12 to 14, 16, 17, 19, 20 and 24 will amplify a few DRB1 alleles.

<sup>5</sup>Short specific PCR fragments are less intense and not as sharp as longer specific bands.

<sup>6</sup>Primer mixes 14 and 16 have a tendency to giving rise to nonspecific amplifications.

<sup>7</sup>Primer mixes 10, 15, 16 and 21 to 23 may give rise to a primer oligomer formation.

<sup>8</sup>Primer mix 12: Specific PCR fragment of 90 bp in the DRB3\*0217 allele. Specific PCR fragment of 145 bp in the DRB3\*0201, 0204 and DRB1\*0337, 1143, 1150 and 1459 alleles.

<sup>9</sup>Primer mix 15: Specific PCR fragment of 120 bp DRB3\*0203, 030101 to 0303 alleles. Specific PCR fragment of 180 bp in the DRB3\*0216 allele. Specific PCR fragment of 210 bp in the DRB3\*0217 allele.

<sup>10</sup>Primer mix 17: Specific PCR fragment of 120 bp in the DRB3\*0108, 0206, 0220 and DRB1\*1367 and 1446 alleles. Specific PCR fragment of 180 bp in the DRB3\*0216 and 0223 alleles.

<sup>11</sup>Primer mix 19: Specific PCR fragment of 180 bp DRB3\*0208, 0218, 0223 and DRB1\*1130 alleles. Specific PCR fragment of 270 bp in the DRB3\*0214 allele.

<sup>12</sup>Primer mix 20: Specific PCR fragment of 165 bp DRB3\*010101 to 0106, 0108 to 0111, 0209, 0210, 030101 to 0303 and DRB1\*1130, 1367 and 1446 alleles. Specific PCR fragment of 180 bp in the DRB3\*0216 allele.

<sup>13</sup>Primer mix 21: Specific PCR fragment of 125 bp in the DRB3\*0110 allele. Specific PCR fragment of 210 bp in the DRB3\*0211 allele.

<sup>14</sup>Primer mix 22: Specific PCR fragment of 185 bp in the DRB3\*0111 allele. Specific PCR fragment of 245 bp in the DRB3\*0212 allele.





## INTERPRETATION TABLE

### DRB3 SSP subtyping

Amplification patterns of the DRB3\*0101 to \*0303 alleles

	Well <sup>5</sup>											
	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	100	125	95	85	95	190	90	120	165	185	270	90
PCR product												145
Length of int.	515	430	515	430	430	430	515	430	430	430	515	515
pos. control <sup>1</sup>												
5'-primer(s) <sup>2</sup>	10	30	11	11	11	8	11	11	10	30	10	51
	<sup>5'</sup> -g CT <sup>3'</sup>	<sup>5'</sup> -gA T <sup>3'</sup>	<sup>5'</sup> -g Cg <sup>3'</sup>	<sup>5'</sup> -Tg T <sup>3'</sup>	<sup>5'</sup> -g Cg <sup>3'</sup>	<sup>5'</sup> -C TC <sup>3'</sup>	<sup>5'</sup> -g Cg <sup>3'</sup>	<sup>5'</sup> -g Cg <sup>3'</sup>	<sup>5'</sup> -g CT <sup>3'</sup>	<sup>5'</sup> -gA C <sup>3'</sup>	<sup>5'</sup> -g CT <sup>3'</sup>	<sup>5'</sup> -g Ag <sup>3'</sup>
			11									
			<sup>5'</sup> -g Tg <sup>3'</sup>									
3'-primer(s) <sup>3</sup>	30	57	28	26	28	57	28	38	51	77	86	67
	<sup>5'</sup> -gTA <sup>3'</sup>	<sup>5'</sup> -C gA <sup>3'</sup>	<sup>5'</sup> -CT g <sup>3'</sup>	<sup>5'</sup> -g gT <sup>3'</sup>	<sup>5'</sup> -CT C <sup>3'</sup>	<sup>5'</sup> -C gA <sup>3'</sup>	<sup>5'</sup> -gTT <sup>3'</sup>	<sup>5'</sup> -CAC <sup>3'</sup>	<sup>5'</sup> -C CC <sup>3'</sup>	<sup>5'</sup> -TA A <sup>3'</sup>	<sup>5'</sup> -C CA <sup>3'</sup>	<sup>5'</sup> -gAA <sup>3'</sup>
		57								77		86
		<sup>5'</sup> -C AA <sup>3'</sup>								<sup>5'</sup> -A gT <sup>3'</sup>		<sup>5'</sup> -C CA <sup>3'</sup>
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
DRB3 allele <sup>4</sup>												
*01010201-010104	1	2	3									
*0102	1	2	3	4								
*0103	1	2			5							
*0104	1	2	3			6						
*0105	1	2	3				7					
*0106	1	2	3					8				
*0107	1		3						9			
*0108	1	2	3					8				
*0109										10		
*0110	1	2	3									
*0111	1	2	3									
*0201									9	10	11	12
*020201-020205									9	10		
*0203									9	10		
*0204									9	10	11	12
*0205	1								9			
*0206									9	10		
*0207									9	10		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE												
DRB3 SSP subtyping												
Amplification patterns of the DRB3*0101 to *0303 alleles												
Well <sup>5</sup>												
13	14	15	16	17	18	19	20	21	22	23	24	
270	185	120	155	120	100	180	165	125	185	195	185	Length of spec.
		180		180		270	180	210	245			PCR product
		210										
515	515	430	515	430	430	515	430	430	430	515	430	Length of int.
												pos. control <sup>1</sup>
10	38	11	38	10	38	10	10	10	9	26	11	5'-primer(s) <sup>2</sup>
5'-g CT <sup>3'</sup>	5'-C gC <sup>3'</sup>	5'-g CT <sup>3'</sup>	5'-C gC <sup>3'</sup>	5'-g CT <sup>3'</sup>	5'-C gC <sup>3'</sup>	5'-g CT <sup>3'</sup>	5'-g CT <sup>3'</sup>	5'-g CT <sup>3'</sup>	5'-Tg C <sup>3'</sup>	5'-gg C <sup>3'</sup>	5'-g CT <sup>3'</sup>	
10	38								29			
5'-g CT <sup>3'</sup>	5'-C gC <sup>3'</sup>								5'-C AT <sup>3'</sup>			
86	86	37	74	37	57	55	51	39	77	77	58	3'-primer(s) <sup>3</sup>
5'-C AC <sup>3'</sup>	5'-C AC <sup>3'</sup>	5'-C gg <sup>3'</sup>	5'-C CC <sup>3'</sup>	5'-gTT <sup>3'</sup>	5'-C gA <sup>3'</sup>	5'-g CA <sup>3'</sup>	5'-CC g <sup>3'</sup>	5'-gCT <sup>3'</sup>	5'-TA A <sup>3'</sup>	5'-TA A <sup>3'</sup>	5'-C CT <sup>3'</sup>	
		37	77	37		57	57	67			60	
		5'-C gA <sup>3'</sup>	5'-A gg <sup>3'</sup>	5'-gTT <sup>3'</sup>		5'-gCT <sup>3'</sup>	5'-C Ag <sup>3'</sup>	5'-gAT <sup>3'</sup>			5'-A gg <sup>3'</sup>	
		57		55		58						
		5'-C Ag <sup>3'</sup>		5'-g CA <sup>3'</sup>		5'-C CT <sup>3'</sup>						
		67		57		86						
		5'-gAA <sup>3'</sup>		5'-C Ag <sup>3'</sup>		5'-C Ag <sup>3'</sup>						
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												DRB3 allele <sup>4</sup>
							20					*01010201-010104
13							20					*0102
13							20					*0103
13							20					*0104
13							20					*0105
13							20					*0106
13	14											*0107
13				17			20					*0108
13	14		16		18		20					*0109
13							20	21				*0110
13							20		22			*0111
												*0201
13	14											*020201-020205
13		15										*0203
			16									*0204
13	14											*0205
13	14			17								*0206
13	14				18							*0207
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

Lot No.: **14F**

Lot-specific Information

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Length of spec.	100	125	95	85	95	190	90	120	165	185	270	90
PCR product												145
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
*0208									9	10		
*0209										10		
*0210										10		
*0211									9	10		
*0212									9	10		
*0213									9	10		
*0214									9	10		
*0215									9			
*0216									9	10		
*0217									9	10		12
*0218									9	10		
*0219									9	10		
*0220									9	10		
*0221									9	10		
*0222									9	10		
*0223									9	10		
*030101-030102	1	2									11	
*0302										10	11	
*0303	1	2										
DRB3 allele <sup>4</sup>												
Well No.	1	2	3	4	5	6	7	8	9	10	11	12
DRB1*0337, 1143, 1459												12
DRB1*0338, 0807, 0819, 0825, 0834, 1331, 1346, 1354, 1377, 1448		w										
DRB1*100101-100102, 1003												
DRB1*1130	w											
DRB1*1144												
DRB1*1149												
DRB1*1150												12
DRB1*1367	1											
DRB1*1446	1				5			8				
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **14F**

Lot-specific Information

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270	185	120	155	120	100	180	165	125	185	195	185	Length of spec. PCR product
		180		180		270	180	210	245			
		210										
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
13	14					19						*0208
13	14				18		20				24	*0209
13	14						20					*0210
13	14							21				*0211
13	14								22			*0212
13	14									23		*0213
						19						*0214
13	14		16									*0215
13	14	15		17			20					*0216
13	14	15										*0217
13	14					19					24	*0218
13	14		16								24	*0219
13				17								*0220
13	14				18						24	*0221
13	14		16									*0222
13	14			17		19						*0223
		15					20				24	*030101-030102
		15					20				24	*0302
13		15					20				24	*0303
												DRB3 allele <sup>4</sup>
13	14	15	16	17	18	19	20	21	22	23	24	Well No.
												DRB1*0337, 1143, 1459
												DRB1*0338, 0807, 0819, 0825, 0834, 1331, 1346, 1354, 1377, 1448
	w											DRB1*100101- 100102, 1003
13						19	20				24	DRB1*1130
			w									DRB1*1144
	w		w									DRB1*1149
			w									DRB1*1150
13				17			20					DRB1*1367
13				17			20					DRB1*1446
13	14	15	16	17	18	19	20	21	22	23	24	Well No.

**Lot No.: 14F**

**Lot-specific Information**

**www.olerup.com**

<sup>1</sup>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 longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB3subtyping.

In addition, wells number 3, 7, 11 to 14, 16, 19 and 23 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.

<sup>2</sup>The codon, in the 2<sup>nd</sup> and 3<sup>rd</sup> exon, matching the specificity-determining 3'-end of the primer is given. Codon numbering as on the [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.

<sup>3</sup>The codon, in the 2<sup>nd</sup> and 3<sup>rd</sup> exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Codon numbering as on the [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.

<sup>4</sup> DRB3\*010101 allele has been shown to be identical to DRB3\*01010201

<sup>5</sup>Primer mix 12: Specific PCR fragment of 90 bp in the DRB3\*0217 allele. Specific PCR fragment of 145 bp in the DRB3\*0201, 0204 and DRB1\*0337, 1143, 1150 and 1459 alleles.

Primer mix 15: Specific PCR fragment of 120 bp DRB3\*0203, 030101 to 0303 alleles. Specific PCR fragment of 180 bp in the DRB3\*0216 allele. Specific PCR fragment of 210 bp in the DRB3\*0217 allele.

Primer mix 17: Specific PCR fragment of 120 bp in the DRB3\*0108, 0206, 0220 and DRB1\*1367 and 1446 alleles. Specific PCR fragment of 180 bp in the DRB3\*0216 and 0223 alleles.

Primer mix 19: Specific PCR fragment of 180 bp DRB3\*0208, 0218, 0223 and DRB1\*1130 alleles. Specific PCR fragment of 270 bp in the DRB3\*0214 allele.

Primer mix 20: Specific PCR fragment of 165 bp DRB3\*010101 to 0106, 0108 to 0111, 0209, 0210, 030101 to 0303 and DRB1\*1130, 1367 and 1446 alleles. Specific PCR fragment of 180 bp in the DRB3\*0216 allele.

Primer mix 21: Specific PCR fragment of 125 bp in the DRB3\*0110 allele. Specific PCR fragment of 210 bp in the DRB3\*0211 allele.

Primer mix 22: Specific PCR fragment of 185 bp in the DRB3\*0111 allele. Specific PCR fragment of 245 bp in the DRB3\*0212 allele.

'w', might be weakly amplified.

CELL LINE VALIDATION SHEET																						
DRB3 SSP subtyping kit																						
					Prod. No.	Well																
						1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
						200851601	200622602	200622603	200622604	200622605	200622606	200622607	200622608	200851609	200622610	200622611	200622612	200622613	200622614	200852615	200852616	
	IHWC cell line	DRB3																				
1	9001 SA					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373	*0202				-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-
5	9009 KAS011					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020 QBL	*0202				-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-
8	9007 DEM					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9067 BTB					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*0202				-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-
17	9008 WILJON					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*0202				-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-
19	9038 BM16	*0202				-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-
20	9059 SLE005	*0301				+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
21	9064 AMALA	*0101				+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
22	9056 KOSE	*0202	*0301			+	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-
23	9124 IHL	*0202				-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-
24	9035 JBUSH	*0202				-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-
25	9049 IBW9					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*0202				-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-
27	9191 CH1007					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*0101				+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
31	9019 DUCAF	*0202				-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-
32	9297 HAG	*0101				+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
33	9098 MT14B					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*0202				-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-
35	9302 SSTO					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB	*0101				+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
38	9099 LZL	*0101				+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
39	9315 CML	*0101				+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
40	9134 WHONP199					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*0301				+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
42	9066 TAB089					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*0201				-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-
45	9239 SHJO					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*0202				-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-
48	9303 TER-ND					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CELL LINE VALIDATION SHEET													
DRB3 SSP subtyping kit													
					Prod. No.	Well							
						17	18	19	20	21	22	23	24
						200852617	200622618	200852619	200622620	200852621	200622622	200622623	200622624
	IHWC cell line	DRB3											
1	9001 SA					-	-	-	-	-	-	-	-
2	9280 LK707					-	-	-	-	-	-	-	-
3	9011 E4181324					-	-	-	-	-	-	-	-
4	9275 GU373	*0202				-	-	-	-	-	-	-	-
5	9009 KAS011					-	-	-	-	-	-	-	-
6	9353 SM					-	-	-	-	-	-	-	-
7	9020 QBL	*0202				-	-	-	-	-	-	-	-
8	9007 DEM					-	-	-	-	-	-	-	-
9	9026 YAR					-	-	-	-	-	-	-	-
10	9107 LKT3					-	-	-	-	-	-	-	-
11	9051 PITOUT					-	-	-	-	-	-	-	-
12	9052 DBB					-	-	-	-	-	-	-	-
13	9067 BTB					-	-	-	-	-	-	-	-
14	9071 OLGA					-	-	-	-	-	-	-	-
15	9075 DKB					-	-	-	-	-	-	-	-
16	9037 SWEIG007	*0202				-	-	-	-	-	-	-	-
17	9008 WILJON					-	-	-	-	-	-	-	-
18	9257 32367	*0202				-	-	-	-	-	-	-	-
19	9038 BM16	*0202				-	-	-	-	-	-	-	-
20	9059 SLE005	*0301				-	-	-	+	-	-	-	+
21	9064 AMALA	*0101				-	-	-	-	-	-	-	-
22	9056 KOSE	*0202	*0301			-	-	-	+	-	-	-	+
23	9124 IHL	*0202				-	-	-	-	-	-	-	-
24	9035 JBUSH	*0202				-	-	-	-	-	-	-	-
25	9049 IBW9					-	-	-	-	-	-	-	-
26	9285 WT49	*0202				-	-	-	-	-	-	-	-
27	9191 CH1007					-	-	-	-	-	-	-	-
28	9320 BEL5GB					-	-	-	-	-	-	-	-
29	9050 MOU					-	-	-	-	-	-	-	-
30	9021 RSH	*0101				-	-	-	+	-	-	-	-
31	9019 DUCAF	*0202				-	-	-	-	-	-	-	-
32	9297 HAG	*0101				-	-	-	+	-	-	-	-
33	9098 MT14B					-	-	-	-	-	-	-	-
34	9104 DHIF	*0202				-	-	-	-	-	-	-	-
35	9302 SSTO					-	-	-	-	-	-	-	-
36	9024 KT17					-	-	-	-	-	-	-	-
37	9065 HHKB	*0101				-	-	-	+	-	-	-	-
38	9099 LZL	*0101				-	-	-	+	-	-	-	-
39	9315 CML	*0101				-	-	-	+	-	-	-	-
40	9134 WHONP199					-	-	-	-	-	-	-	-
41	9055 H0301	*0301				-	-	-	+	-	-	-	+
42	9066 TAB089					-	-	-	-	-	-	-	-
43	9076 T7526					-	-	-	-	-	-	-	-
44	9057 TEM	*0201				-	-	-	-	-	-	-	-
45	9239 SHJO					-	-	-	-	-	-	-	-
46	9013 SCHU					-	-	-	-	-	-	-	-
47	9045 TUBO	*0202				-	-	-	-	-	-	-	-
48	9303 TER-ND					-	-	-	-	-	-	-	-



## CERTIFICATE OF ANALYSIS

### **Olerup SSP<sup>®</sup> DRB3 SSP**

**Product number:** 101.121-24/04 – including *Taq* pol.  
**Lot number:** 14F  
**Expiry date:** 2010-October-01  
**Number of tests:** 24 tests – Product No. 101.121-24  
4 tests – Product No. 101.121-04  
**Number of wells per test:** 24

#### **Well specifications:**

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2008-516-01	9	2008-516-09	17	2008-516-17
2	2006-226-02	10	2006-226-10	18	2006-226-18
3	2006-226-03	11	2006-226-11	19	2008-516-19
4	2006-226-04	12	2006-226-12	20	2006-226-20
5	2006-226-05	13	2006-226-13	21	2008-516-21
6	2006-226-06	14	2006-226-14	22	2006-226-22
7	2006-226-07	15	2008-516-15	23	2006-226-23
8	2006-226-08	16	2008-516-16	24	2006-226-24

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

No DNAs carrying the alleles to be amplified by primer solutions 4 to 8 and 15 to 24 were available. The specificities of the primers in primer solutions 5, 8, 16 to 19 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 4, 6, 15, 20, 22 and 23 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solutions 7 and 21 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. One of the 3'-primers in primer solutions 2 and 12 were not possible to test. One of the 5'-primers in primer solution 3 was not possible to test.

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

**Date of approval:** 2009-May-25

**Approved by:**

**Quality Control, Supervisor**

Lot No.: **14F**

Lot-specific Information

[www.olerup.com](http://www.olerup.com)

## Declaration of Conformity

**Product name:** *Olerup* SSP® DRB3  
**Product number:** 101.121-24/04  
**Lot number:** 14F

**Intended use:** DRB3 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 9001:2000 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  
2009-May-25

Olle Olerup  
Managing Director



Lot No.: **14F**

Lot-specific Information

[www.olerup.com](http://www.olerup.com)

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**Fax:** +46-8-717 88 18

**E-mail:** [info-ssp@olerup.com](mailto:info-ssp@olerup.com)

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**Distributed by:**

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**Tel:** +43-1-710 15 00

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**E-mail:** [support-at@olerup.com](mailto:support-at@olerup.com)

**Web page:** <http://www.olerup.com>

**Olerup Inc.**, 901 S. Bolmar St., Suite R, West Chester, PA 19382

**Tel:** 1-877-OLERUP1

**Fax:** 610-344-7989

**E-mail:** [info.us@olerup.com](mailto:info.us@olerup.com)

**Web page:** <http://www.olerup.com>

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.