Olerup SSP® DRB3

Product number:	**101.121-24/04 – including *Taq* pol.**
Lot number:	**52G**
Expiry date:	**2011-November-01**
Number of tests:	**24 tests – Product No. 101.121-24**
	4 tests – Product No. 101.121-04
Number of wells per test:	**27**
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. 52G.

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. 14F)** was made.

Three wells have been added to the **DRB3** kit,
wells **25 to 27**.

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	-	Added	Primer added for the DRB3*010105 allele.
5	-	Added	Primer for the DRB3*0110 allele, from well 21.
10	-	-	Exchanged positive control primer pair to reduce dimer formation.
16	-	-	Exchanged positive control primer pair to reduce dimer formation.
21	-	Moved, added	Primer moved to well 5. Primer added for the DRB3*0113 allele.
23	-	-	Exchanged positive control primer pair to reduce dimer formation.

25	New	New	New primer pair for the DRB3*0112 allele.
26	New	New	New primer pair for the DRB3*0114 allele.
27	New	New	New primer pair for improved resolution of the DRB3*0201 and DRB3*0224 alleles.

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 27 PCR reactions in a 32 well cut PCR plate. Wells 28 to 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**	**empty**	**empty**	**empty**	**empty**	**empty**

The 32 well cut PCR plate is marked with 'DRB3' in silver/gray ink.
Well No. 1 is marked with the Lot No. '52G'.
A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.
The PCR plates are covered with a PCR-compatible foil.
Please note: When removing each 32 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION
The interpretation of DRB3 subtypings will be influenced by twelve DRB1*03 alleles, the DRB1*0482, four DRB1*08 alleles, the DRB1*10, seven DRB1*11 alleles, eight DRB1*13 alleles and seven DRB1*14 alleles. 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*0114, DRB3*0201 to DRB3*0225 and DRB3*0301 to DRB3*0303**, recognized by the HLA Nomenclature Committee in October 2009[1] 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*010105 alleles or the DRB3*020201 to DRB3*020205 alleles or the DRB3*030101 to DRB3*030103 alleles.

[1]DRB alleles listed on the IMGT/HLA web page 2009-October-19, release 2.27.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 42 phenotypically different DRB3 alleles can be combined in 903 homozygous and heterozygous combinations. 298 of these genotypes do not give rise to unique amplification patterns. (The different sizes of the specific PCR products generated by primer mixes 5, 12, 15, 17 and 19 to 22 were not considered in these calculations.)

```
++++++-- ----+--- ---+---- ---    0102,0103 = 0102,0110
+++++--- +---++-- ---+---- ---    0102,0107 = 0102,0205
+++++--- ----+--- ---+---- ---    0101,0102 = 0102,0102
+++-++-- ----+--- ---+---- ---    0103,0104 = 0104,0110
+++-+-+- ----+--- ---+---- ---    0103,0105 = 0105,0110
+++-+--+ ----+--- +--+---- ---    0103,0108 = 0108,0110
+++-+--+ ----+--- ---+---- ---    0103,0106 = 0106,0110
+++-+--- +---++-- ---+---- ---    0103,0107 = 0107,0110 = 0110,0205
+++-+--- ----+--- ----+--- ---    0103,0113 = 0110,0113
+++-+--- ----+--- ----+-- ---    0103,0111 = 0110,0111
+++-+--- ----+--- ---+---- +--    0103,0112 = 0110,0112
+++-+--- ----+--- ---+---- ---    0101,0103 = 0101,0110 = 0103,0110
                                  = 0110,0110
+++--+-- +---++-- ---+---- ---    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
+++---- ++--++-- ---+++-- ---    0111,0211 = 0113,0212
+++---- ++--++-- ----++-- ---    0101,0211 = 0113,0202 = 0113,0211
+++---- ++--++-- ----+-+- ---    0101,0212 = 0111,0202 = 0111,0212
+++---- +---++-- ----++-- ---    0107,0113 = 0113,0205
+++---- +---++-- ----+-+- ---    0107,0111 = 0111,0205
+++---- +---++-- ---+---- +--    0107,0112 = 0112,0205
+++---- +---++-- ---+---- ---    0101,0107 = 0101,0205
+++---- ----+--- ----++-- ---    0101,0113 = 0113,0113
+++---- ----+--- ----+-+- ---    0101,0111 = 0111,0111
+++---- ----+--- ---+---- +--    0101,0112 = 0112,0112
++----- +++-++++ ---+---+ ---    0219,0301 = 0222,0301
++----- +++-+++- +--+---+ ---    0206,0301 = 0216,0301
++----- +++-+++- -+-+---+ ---    0207,0301 = 0221,0301
++----- +++-+++- --++---+ ---    0208,0301 = 0218,0301
++----- +++-+++- ---+---+ ---    0202,0301 = 0225,0301
++----- ++--++++ ---+---+ ---    0219,0303 = 0222,0303
++----- ++--+++- +--+---+ ---    0206,0303 = 0216,0303
++----- ++--+++- -+-+---+ ---    0207,0303 = 0221,0303
++----- ++--+++- --++---+ ---    0208,0303 = 0218,0303
++----- ++--+++- ---+---+ ---    0202,0303 = 0225,0303
+-+---- ++--++-- +------- ---    0107,0206 = 0107,0220
+-+---- ++--++-- --+---- ---    0107,0208 = 0107,0214
+-+---- +---++-- -------- ---    0107,0107 = 0107,0205
```

```
+------- ++--++-- +------- ---    0205,0206 = 0205,0220
+------- ++--++-- --+----- ---    0205,0208 = 0205,0214
-------- ++++++-+ -+-+---- ---    0109,0204 = 0109,0224
-------- ++++++-+ -------+ ---    0204,0219 = 0204,0225 = 0219,0224
-------- ++++++-+ -------- --+    0201,0215 = 0201,0222
-------- ++++++-+ -------- ---    0202,0204 = 0204,0215 = 0204,0222
                                  = 0215,0224 = 0222,0224
-------- +++---+ -------- ---    0204,0204 = 0204,0224
-------- +++---- -------- --+    0201,0201 = 0201,0224
-------- +++-++++ ---+---+ ---    0215,0302 = 0219,0302 = 0222,0302
-------- +++-+++- +--+---+ ---    0206,0302 = 0216,0302
-------- +++-+++- -+-+---+ ---    0207,0302 = 0221,0302
-------- +++-+++- --++---+ ---    0208,0302 = 0218,0302
-------- +++-+++- ---+---+ ---    0202,0302 = 0225,0302
-------- ++-+++++ -------- ---    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 = 0109,0225
                                  = 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 = 0215,0225
                                  = 0219,0219 = 0219,0222 =
                                  0219,0225 = 0222,0225
-------- ++--++-+ -------- ---    0202,0215 = 0202,0222 = 0215,0222
                                  = 0222,0222
-------- ++--++-- ++-+---+ ---    0206,0209 = 0209,0220
-------- ++--++-- ++-----+ ---    0206,0221 = 0220,0221
-------- ++--++-- ++------ ---    0206,0207 = 0207,0220
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-------- ++--++-- +-+----+ ---	0206,0218 = 0218,0220 = 0218,0223 = 0223,0225
-------- ++--++-- +-+----- ---	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
-------- ++--++-- +------+ ---	0206,0225 = 0220,0225
-------- ++--++-- +------- ---	0202,0206 = 0202,0220 = 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 = 0209,0225 = 0210,0221
-------- ++--++-- -+-----+ ---	0202,0221 = 0207,0221 = 0207,0225 = 0221,0221 = 0221,0225
-------- ++--++-- -+------ ---	0202,0207 = 0207,0207
-------- ++--++-- --++---- ---	0208,0210 = 0210,0214
-------- ++--++-- --+-+--- ---	0208,0211 = 0211,0214
-------- ++--++-- --+--+-- ---	0208,0212 = 0212,0214
-------- ++--++-- --+---+- ---	0208,0213 = 0213,0214
-------- ++--++-- --+----+ ---	0202,0218 = 0208,0218 = 0208,0225 = 0214,0218 = 0214,0225 = 0218,0218 = 0218,0225
-------- ++--++-- --+----- ---	0202,0208 = 0202,0214 = 0208,0208 = 0208,0214
-------- ++--++-- ----+--- ---	0202,0211 = 0211,0211
-------- ++--++-- -----+-- ---	0202,0212 = 0212,0212
-------- ++--++-- ------+- ---	0202,0213 = 0213,0213
-------- ++--++-- -------+ ---	0202,0225 = 0225,0225
-------- -+--++-+ -+-+---- ---	0109,0109 = 0109,0210
-------- -+--++-- -+-+---+ ---	0209,0209 = 0209,0210

0101 = 01010201-010105
0202 = 020201-020205
0301 = 030101-030103

SPECIFICITY TABLE

DRB3 SSP subtyping

Specificities and sizes of the PCR products of the 27 primer mixes used for DRB3 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified DRB3 alleles[3]	Amplified DRB1[4] alleles
1[5]	100 bp	**515 bp**	*01010201-0108, 0110-0113, 0205, 030101-030103, 0303	*0342, 1130[w], 1367, 1446
2[5]	125 bp	430 bp	*01010201-0106, 0108, 0110-0114, 030101-030103, 0303	*0338[w], 0482[w], 0807[w], 0819[w], 0825[w], 0834[w], 1331[w], 1346[w], 1354[w], 1377[w], 1448[w]
3[5]	95 bp	**515 bp**	*01010201-0102, 0104-0108, 0110-0113	*0342
4[5]	85 bp	430 bp	*0102	
5[5,8]	95 bp, 125 bp	430 bp	*0103, 0110	*1446
6	190 bp	430 bp	*0104	
7[5]	90 bp	**515 bp**	*0105	
8[5]	120 bp	430 bp	*0106, 0108	*0342, 1446
9[6,7]	165 bp	430 bp	*0107, 0201-0208, 0211-0225	
10[6,7]	185 bp	**515 bp**	*0109, 0201-0204, 0206-0214, 0216-0225, 0302	
11[6]	270 bp	**515 bp**	*0201, 0204, 0224, 030101-0302	*0342
12[5,9]	90 bp, 145 bp	**515 bp**	*0201, 0204, 0217, 0224	*0337, 1143, 1150, 1459, 1486
13[6]	270 bp	**515 bp**	*01010201-0113, 020201-0203, 0205-0213, 0215-0223, 0225, 0303	*1130, 1367, 1446

14[6]	185 bp	**515 bp**	*0107, 0109, 020201-020205, 0205-0213, 0215-0219, 0221-0223, 0225	*100101[W]-100103[W], 1003[W]
15[5,7,10]	120 bp, 180 bp, 210 bp	430 bp	*0203, 0216, 0217, 030101-0303	
16[6,7]	155 bp	430 bp	*0109, 0204, 0215, 0219, 0222	*0344, 100101[W]-1003[W], 1144[W], 1149[W], 1150[W]
17[5,11]	120 bp, 180 bp	430 bp	*0108, 0206, 0216, 0220, 0223	*0342, 1367, 1446
18[5]	100 bp	430 bp	*0109, 0207, 0209, 0221	
19[12]	180 bp, 270 bp	**515 bp**	*0208, 0214, 0218, 0223	*1130
20[13]	165, 180 bp	430 bp	*01010201-0106, 0108-0113, 0209, 0210, 0216, 030101-0303	*0342, 1130, 1367, 1446
21[5,7,14]	120 bp, 210 bp	430 bp	*0113, 0211	
22[7,15]	185 bp, 245 bp	430 bp	*0111, 0212	
23[7]	195 bp	430 bp	*0213	
24	185 bp	430 bp	*0209, 0218, 0219, 0221, 0225, 030101-0303	*1130
25	260 bp	430 bp	*0112	
26	235 bp	430 bp	*0114	*030102, 030202, 030503, 031302, 0317, 0324, 0327, 0335, 112701, 1184, 133302, 133303, 1361, 1438, 1447, 1450
27[5]	90 bp	430 bp	*0201	

[1]Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of 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 lengths of the specific PCR product(s) of the alleles amplified by these primer mixes are given.
Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.
PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.
PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.
Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherit feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.
[2]The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 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 DRB3 subtyping.
In addition, wells number 3, 7, 10 to 14 and 19 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.
[3]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.
[4]Due to the sharing of sequence motifs between DRB3 and DRB1 alleles, primer mixes 1 to 3, 5, 8, 11 to 14, 16, 17, 19, 20, 24 and 26 will amplify a few DRB1 alleles.
[5]Short specific PCR fragments are less intense and not as sharp as longer specific bands.
[6]Primer mixes 9, 10, 11, 13, 14 and 16 have a tendency to giving rise to nonspecific amplifications.
[7]Primer mixes 9, 10, 15, 16 and 21 to 23 may give rise to a primer oligomer formation.
[8]Primer mix 5: Specific PCR fragment of 95 bp in the DRB3*0103 and the DRB1*1446 alleles. Specific PCR fragment of 125 bp in the DRB3*0110 allele.
[9]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 0224 and the DRB1*0337, 1143, 1150, 1459 and 1486 alleles.
[10]Primer mix 15: Specific PCR fragment of 120 bp DRB3*0203 and 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.
[11]Primer mix 17: Specific PCR fragment of 120 bp in the DRB3*0108, 0206, 0220 and DRB1* DRB1*0342, 1367 and 1446 alleles. Specific PCR fragment of 180 bp in the DRB3*0216 and 0223 alleles.
[12]Primer mix 19: Specific PCR fragment of 180 bp in the DRB3*0208, 0218, 0223 and DRB1*1130 alleles. Specific PCR fragment of 270 bp in the DRB3*0214 allele.
[13]Primer mix 20: Specific PCR fragment of 165 bp in the DRB3*01010201-0106, 0108-0113, 0209, 0210 and 030101-0303 and in the DRB1*0342, DRB1*1130, DRB1*1367 and DRB1*1446 alleles. Specific PCR fragment of 180 bp in the DRB3*0216 allele.
[14]Primer mix 21: Specific PCR fragment of 120 bp in the DRB3*0113 allele. Specific PCR fragment of 210 bp in the DRB3*0211 allele.
[15]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.

INTERPRETATION TABLE

DRB3 SSP subtyping

Amplification patterns of the DRB3*0101 to *0303 alleles

	Well[5]															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Length of spec.	**100**	**125**	**95**	**85**	**95**	**190**	**90**	**120**	**165**	**185**	**270**	**90**	**270**	**185**	**120**	**155**
PCR product					**125**							**145**			**180**	
															210	
Length of int.	**515**	**430**	**515**	**430**	**430**	**430**	**515**	**430**	**430**	**515**	**515**	**515**	**515**	**515**	**430**	**430**
pos. control[1]																
5'-primer(s)[2]	5'-gCT' 10(116)	5'-gAT' 30(175)	5'-gCg' 11(119)	5'-TgT' 11(118)	5'-gCg' 11(119)	5'-CTC' 8(110)	5'-gCg' 11(119)	5'-gCg' 11(119)	5'-gCT' 10(116)	5'-gAC' 30(175)	5'-gCT' 10(116)	5'-gAg' 51(239)	5'-gCT' 10(116)	5'-CgC' 38(200)	5'-gCT' 11(119)	5'-CgC' 38(200)
			5'-gTg' 11(119)						5'-gCT' 10(116)				5'-gCT' 10(116)	5'-CgC' 38(200)		
3'-primer(s)[3]	5'-gTA' 30(175)	5'-CgA' 57(257)	5'-CTg' 28(171)	5'-ggT' 26(164)	5'-CTC' 28(171)	5'-CgA' 57(257)	5'-gTT' 28(169)	5'-CAC' 38(199)	5'-CCC' 51(239)	5'-AgT' 77(317)	5'-CCA' 86(344)	5'-gAA' 67(286)	5'-CAC' 86(344)	5'-CAC' 86(344)	5'-Cgg' 37(197)	5'-CCC' 74(308)
	5'-gTA' 30(175)	5'-CAA' 57(257)	5'-ATg' 28(171)		5'-gCT' 39(202)					5'-TAA' 77(318)		5'-CCA' 86(344)			5'-CgA' 37(197)	5'-Agg' 77(317)
															5'-CAg' 57(257)	
															5'-gAA' 67(286)	
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**

INTERPRETATION TABLE

DRB3 SSP subtyping

Amplification patterns of the DRB3*0101 to *0303 alleles

Well[5]											
17	18	19	20	21	22	23	24	25	26	27	
120	100	180	165	120	185	195	185	260	235	90	Length of spec.
180		270	180	210	245						PCR product
430	430	515	430	430	430	430	430	430	430	430	Length of int.
											pos. control[1]
5'-gCT-3' 10(116)	5'-CgC-3' 38(200)	5'-gCT-3' 10(116)	5'-gCT-3' 10(116)	5'-gCT-3' 10(116)	5'-TgC-3' 9(112)	5'-ggC-3' 26(163)	5'-gCT-3' 11(119)	5'-gCT-3' 10(116)	5'-TAC-3' 12(122)	5'-CAT-3' 164(577	5'-primer(s)[2]
					5'-CAT-3' 29(173)						
5'-gTT-3' 37(196)	5'-CgA-3' 57(257)	5'-gCA-3' 55(251)	5'-CCg-3' 51(239)	5'-gAg-3' 37(196)	5'-TAA-3' 77(318)	5'-TAA-3' 77(318)	5'-CCT-3' 58(260)	5'-CCg-3' 84(337)	5'-AAT-3' 77(317)	5'-ACg-3' 179(624	3'-primer(s)[3]
5'-gTT-3' 37(196)		5'-gCT-3' 57(256)	5'-CAg-3' 57(257)	5'-gAT-3' 67(286)			5'-Agg-3' 60(266)				
5'-gCA-3' 55(251)		5'-CCT-3' 58(260)									
5'-CAg-3' 57(257)		5'-CAg-3' 86(344)									
17	18	19	20	21	22	23	24	25	26	27	Well No.

Length of spec.	**100**	**125**	**95**	**85**	**95**	**190**	**90**	**120**	**165**	**185**	**270**	**90**	**270**	**185**	**120**	**155**
PCR product					**125**							**145**			**180**	
															210	
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
DRB3 allele[4]																
***01010201-010105**	**1**	**2**	**3**										**13**			
***0102**	**1**	**2**	**3**	**4**									**13**			
***0103**	**1**	**2**			**5**								**13**			
***0104**	**1**	**2**	**3**			**6**							**13**			
***0105**	**1**	**2**	**3**				**7**						**13**			
***0106**	**1**	**2**	**3**					**8**					**13**			
***0107**	**1**		**3**						**9**				**13**	**14**		
***0108**	**1**	**2**	**3**					**8**					**13**			
***0109**										**10**			**13**	**14**		**16**
***0110**	**1**	**2**	**3**		**5**								**13**			
***0111**	**1**	**2**	**3**										**13**			
***0112**	**1**	**2**	**3**										**13**			
***0113**	**1**	**2**	**3**										**13**			
***0114**		**2**														
***0201**									**9**	**10**	**11**	**12**				
***020201-020205**									**9**	**10**			**13**	**14**		
***0203**									**9**	**10**			**13**		**15**	
***0204**									**9**	**10**	**11**	**12**				**16**
***0205**	**1**								**9**				**13**	**14**		
***0206**									**9**	**10**			**13**	**14**		
***0207**									**9**	**10**			**13**	**14**		
***0208**									**9**	**10**			**13**	**14**		
***0209**										**10**			**13**	**14**		
***0210**										**10**			**13**	**14**		
***0211**									**9**	**10**			**13**	**14**		
***0212**									**9**	**10**			**13**	**14**		
***0213**									**9**	**10**			**13**	**14**		
***0214**									**9**	**10**						
***0215**									**9**				**13**	**14**		**16**
***0216**									**9**	**10**			**13**	**14**	**15**	
***0217**									**9**	**10**		**12**	**13**	**14**	**15**	
***0218**									**9**	**10**			**13**	**14**		
***0219**									**9**	**10**			**13**	**14**		**16**
***0220**									**9**	**10**			**13**			
***0221**									**9**	**10**			**13**	**14**		
***0222**									**9**	**10**			**13**	**14**		**16**
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**

120	100	180	165	120	185	195	185	260	235	90	Length of spec.
180		270	180	210	245						PCR product
17	18	19	20	21	22	23	24	25	26	27	Well No.
											DRB3 allele[4]
			20								*01010201-010105
			20								*0102
			20								*0103
			20								*0104
			20								*0105
			20								*0106
											*0107
17			20								*0108
	18		20								*0109
			20								*0110
			20		22						*0111
			20					25			*0112
			20	21							*0113
									26		*0114
										27	*0201
											*020201-020205
											*0203
											*0204
											*0205
17											*0206
	18										*0207
		19									*0208
	18		20				24				*0209
			20								*0210
				21							*0211
					22						*0212
						23					*0213
		19									*0214
											*0215
17			20								*0216
											*0217
		19					24				*0218
							24				*0219
17											*0220
	18						24				*0221
											*0222
17	18	19	20	21	22	23	24	25	26	27	Well No.

Length of spec.	**100**	**125**	**95**	**85**	**95**	**190**	**90**	**120**	**165**	**185**	**270**	**90**	**270**	**185**	**120**	**155**
PCR product					**125**							**145**			**180**	
															210	
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
***0223**									**9**	**10**			**13**	**14**		
***0224**									**9**	**10**	**11**	**12**				
***0225**									**9**	**10**			**13**	**14**		
***030101-030103**	**1**	**2**									**11**				**15**	
***0302**										**10**	**11**				**15**	
***0303**	**1**	**2**											**13**		**15**	
DRB1*030102, 030202, 030503, 031302, 0317, 0324, 0327, 0335, 112701, 1184, 133302, 133303, 1361, 1438, 1447, 1450																
DRB1*0337, 1143, 1459, 1486												**12**				
DRB1*0338, 0482, 0807, 0819, 0825, 0834, 1331, 1346, 1354, 1377, 1448		**w**														
***DRB1*0342**	**1**		**3**					**8**			**11**					
***DRB1*0344**																**16**
DRB1*100101-100103, 1003														**w**		**w**
DRB1*1002, 1144, 1149																**w**
***DRB1*1130**	**w**												**13**			
***DRB1*1150**												**12**				**w**
***DRB1*1367**	**1**												**13**			
***DRB1*1446**	**1**				**5**			**8**					**13**			
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**

120	100	180	165	120	185	195	185	260	235	90	Length of spec.
180		270	180	210	245						PCR product
17	18	19	20	21	22	23	24	25	26	27	Well No.
17		19									*0223
											*0224
							24				*0225
			20				24				*030101-030103
			20				24				*0302
			20				24				*0303
									26		*DRB1*030102, 030202, 030503, 031302, 0317, 0324, 0327, 0335, 112701, 1184, 133302, 133303, 1361, 1438, 1447, 1450*
											*DRB1*0337, 1143, 1459, 1486*
											*DRB1*0338, 0482, 0807, 0819, 0825, 0834, 1331, 1346, 1354, 1377, 1448*
17			20								*DRB1*0342*
											*DRB1*0344*
											*DRB1*100101-100103, 1003*
											*DRB1*1002, 1144, 1149*
		19	20				24				*DRB1*1130*
											*DRB1*1150*
17			20								*DRB1*1367*
17			20								*DRB1*1446*
17	18	19	20	21	22	23	24	25	26	27	Well No.

[1]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 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 DRB3subtyping.
In addition, wells number wells number 3, 7, 10 to 14 and 19 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.
[2]The codon, and in parenthesis the nucleotide, in the 2nd and 3rd 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.
[3]The codon, and in parenthesis the nucleotide, in the 2nd and 3rd 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.
[4]DRB3*010101 allele has been shown to be identical to DRB3*01010201
[5]Primer mix 5: Specific PCR fragment of 95 bp in the DRB3*0103 and the DRB1*1446 alleles. Specific PCR fragment of 125 bp in the DRB3*0110 allele.
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 0224 and the DRB1*0337, 1143,1150, 1459 and 1486 alleles.
Primer mix 15: Specific PCR fragment of 120 bp DRB3*0203 and 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* DRB1*0342, 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 in the 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 in the DRB3*01010201-0106, 0108-0113, 0209, 0210 and 030101-0303 and in the DRB1*0342, DRB1*1130, DRB1*1367 and DRB1*1446 alleles. Specific PCR fragment of 180 bp in the DRB3*0216 allele.
Primer mix 21: Specific PCR fragment of 120 bp in the DRB3*0113 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

					Well													
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.	200966001	200622602	200622603	200622604	200966005	200622606	200622607	200622608	200851609	200966010	200622611	200622612	200622613	200622614	200851615	200966016
	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	9025	DEU			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*0202		-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
17	9282	CTM3953540	*0101		+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-
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

					Well										
					17	18	19	20	21	22	23	24	25	26	27
				Prod. No.	200852617	200622618	200851619	200622620	200966021	200622622	200966023	200622624	200966025	200966026	200966027
	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	9025	DEU			-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR			-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3			-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT			-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB			-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM			-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA			-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB			-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*0202		-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*0101		-	-	-	-	-	-	-	-	-	-	-
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® DRB3 SSP

Product number: **101.121-24/04 – including *Taq* pol.**
Lot number: **52G**
Expiry date: **2011-November-01**
Number of tests: **24 tests – Product No. 101.121-24**
4 tests – Product No. 101.121-04
Number of wells per test: **27**

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2009-660-01	11	2006-226-11	21	2009-660-21
2	2006-226-02	12	2006-226-12	22	2006-226-22
3	2006-226-03	13	2006-226-13	23	2009-660-23
4	2006-226-04	14	2006-226-14	24	2006-226-24
5	2009-660-05	15	2008-516-15	25	2009-660-25
6	2006-226-06	16	2009-660-16	26	2009-660-26
7	2006-226-07	17	2008-516-17	27	2009-660-27
8	2006-226-08	18	2006-226-18		
9	2008-516-09	19	2008-516-19		
10	2009-660-10	20	2006-226-20		

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, 16 to 19, 21 to 23 and 25 to 27 were available. The specificities of the primers in primer solutions 5, 8, 16 to 19, 21 and 26 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 4, 6, 22, 23 and 27 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solutions 7 and 25 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. One or two of the 3'-primers in primer solutions 1, 5, 12, 15, 17, 19 and 21 were not possible to test. In primer solutions 2 and 3 one of the 5'-primers and one of the 3'-primers was not possible to test. Additional 3'-primers in primer solutions 15, 20 and 24 were tested by separately adding one 5'-primer.

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

Date of approval: 2009- November-11

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DRB3
Product number: 101.121-24/04
Lot number: 52G

Intended use: DRB3 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2008 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex 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-November-11

Olle Olerup
Managing Director

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