Olerup SSP® DRB1*03

Product number:	**101.113-24/04 – including *Taq* pol.** **101.113-24u/04u – without *Taq* pol.**
Lot number:	**36E**
Expiry date:	**2010-March-01**
Number of tests:	**24 tests – Product No. 101.113-24** **4 tests – Product No. 101.113-04**
Number of wells per test:	**29**
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. 36E.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® DRB1*03 LOT

The DRB1*03 specificity and interpretation tables have been updated for the DRB alleles described since the previous *Olerup* SSP® DRB1*03 lot was made **(Lot No. X31)**.

Five wells have been added to the DRB1*03 kit,
wells **25 to 29**.

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

Well	5'-primer	3'-primer	rationale
2	-	Removed	Primer moved to new vial 26.
18	-	Modified	Primer modified for the DRB1*030104 allele.
19	Exchanged	-	Primer exchanged for less primer dimer formation tendency.
25	New	New	New primer pair for the DRB1*0332 allele.
26	New	New	Primer pair from vial 2.
27	New	New	New primer pair for the DRB1*0334 allele.
28	New	New	New primer pair for the DRB1*0336 allele.
29	New	New	New primer pair for the DRB1*0339 allele.

Change in revision R01 compared to R00:

1. Footnote has been to the Specificity Table for primer mix 28.

PRODUCT DESCRIPTION

DRB1*03 SSP subtyping

CONTENT
The primer set contains 5'- and 3'-primers for identifying the DRB1*0301 to DRB1*0339 alleles.

PLATE LAYOUT
Each test consists of 29 PCR reactions in a 32 well cut PCR plate. Wells 30 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**	**28**	**29**	**empty**	**empty**	**empty**

The 24 well cut PCR plate is marked with 'DRB1*03'.
Well No. 1 is marked with the Lot No. '36E'.
The PCR plates are heat-sealed with a PCR-compatible foil.
Please note: When removing each 32 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION
The interpretation of DRB1*03 PCR-SSP subtypings will be influenced by one DRB1*01 allele, one, DRB1*04 allele, one DRB1*08 allele, two DRB1*09 alleles, several DRB1*11 alleles, three DRB1*12 alleles, one DRB1*15 allele, one DRB1*16 allele, as well as by many DRB1*13 and DRB1*14 alleles, when present on the other haplotype and also by the DRB3*0107, DRB3*0108, DRB3*0109, DRB3*0204, DRB3*0206, DRB3*0219, DRB3*0220, DRB3*0222 and DRB3*0303 alleles.

UNIQUELY IDENTIFIED ALLELES
All the phenotypically different DRB1*03 alleles, i.e. **DRB1*0301 to DRB1*0339**, recognized by the HLA Nomenclature Committee in January 2008[1] will give rise to unique amplification patterns by the primers in the DRB1*03 subtyping kit.
The DRB1*03 subtyping kit cannot distinguish the DRB1*030101 and 030106 alleles, the DRB1*030201 and 030202 alleles or the DRB1*030501 and 030502 alleles.

[1]DRB1 alleles listed on the IMGT/HLA web page 2008-January-11, release 2.20.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 39 DRB1*03 alleles can be combined in 780 homozygous and heterozygous combinations. 210 of these genotypes do not give rise to unique amplification patterns. The different sizes of the specific PCR products generated by the primers in vials 11, 14, 20 and 23 have not been considered in these calculations.

```
++++-+-+ ++------ -+----++ ----- 0302,0323 = 0319,0338 = 0323,0338
++++-+-+ ++----- -+-----+ ----- 0301,0338 = 0302,0313 = 0313,0338
++-+-+-+ ++------ -+-+---+ ----- 0303,0325 = 0303,0328
+-++-+-- +-+----+ -+----+- ----- 0309,0315 = 0314,0323
+-++-+-- +-+----- -+----+- ----- 0301,0309 = 0305,0323 = 0309,0313 =
                                  0309,0319 = 0309,0323
+-++-+-- +------+ -+------ ----- 0301,0314 = 0305,0315 = 0314,0315
+--+++-- +---+--- -+-+---- ----- 0304,0311 = 0304,0324
+--+++-- +------- -+-+---- ----- 0301,0304 = 0304,0328
+--+-++- +------- -+-+---- ----- 0308,0325 = 0308,0328
+--+-+-+ +------- -+-+---- ----- 0307,0325 = 0307,0328
+--+-+-+ +------- -+--+--- ----- 0301,0321 = 0307,0330
+--+-+-- ++--+-+- -+------ ----- 0301,0317 = 0317,0337 = 0335,0337
+--+-+-- ++--+--- -------- ----- 0306,0311 = 0311,0335
+--+-+-- ++---+-- -+------ -+--- 0312,0326 = 0316,0326
+--+-+-- ++---+-- -+------ ----- 0306,0312 = 0306,0316
+--+-+-- ++------ -+-+---- -+--- 0325,0326 = 0326,0328 = 0331,0333
+--+-+-- ++------ -+-+---- ----- 0301,0331 = 0306,0325 = 0306,0328 =
                                  0328,0331
+--+-+-- ++------ -+------ -+--- 0301,0326 = 0306,0333 = 0326,0333
+--+-+-- +-+-+--- -+----+- ----- 0311,0323 = 0323,0324
+--+-+-- +-+-+--- -+------ ----- 0311,0313 = 0313,0324
+--+-+-- +-+--+-- -+----+- ----- 0312,0323 = 0316,0323
+--+-+-- +-+--+-- -+------ ----- 0312,0313 = 0313,0316
+--+-+-- +-+----- -+-+--+- ----- 0323,0325 = 0323,0328
+--+-+-- +-+----- -+-+---- ----- 0313,0325 = 0313,0328
+--+-+-- +-+----- -+----+- ----- 0301,0323 = 0313,0319 = 0313,0323 =
                                  0319,0323 = 0323,0323
+--+-+-- +-+----- -+------ ----- 0301,0313 = 0313,0313
+--+-+-- +--+---- -+-+---- ----- 0310,0325 = 0310,0328
+--+-+-- +---++-- -+------ ----- 0311,0312 = 0311,0316
+--+-+-- +---+-+- -+------ ----- 0311,0337 = 0324,0337
+--+-+-- +---+--+ -+------ ----- 0311,0315 = 0315,0324
+--+-+-- +---+--- ++------ ----- 0311,0320 = 0320,0324
+--+-+-- +---+--- -++----- ----- 0311,0322 = 0322,0324
+--+-+-- +---+--- -+-+---- ----- 0311,0325 = 0311,0328 = 0324,0325 =
                                  0324,0328
+--+-+-- +---+--- -+--+--- ----- 0311,0330 = 0324,0330
+--+-+-- +---+--- -+---+-- ----- 0311,0318 = 0318,0324
+--+-+-- +---+--- -+----+- ----- 0311,0319 = 0319,0324
+--+-+-- +---+--- -+------ +---- 0311,0332 = 0324,0332
+--+-+-- +---+--- -+------ -+--- 0311,0333 = 0324,0333
+--+-+-- +---+--- -+------ --+-- 0311,0334 = 0324,0334
+--+-+-- +---+--- -+------ ---+- 0311,0336 = 0324,0336
+--+-+-- +---+--- -+------ ----+ 0311,0339 = 0324,0339
+--+-+-- +---+--- -+------ ----- 0301,0311 = 0301,0324
+--+-+-- +---+--- -------- ----- 0311,0311 = 0311,0324
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+--+-+-- +----++- -+------ ----- 0312,0337 = 0316,0337
+--+-+-- +----+-+ -+------ ----- 0312,0315 = 0315,0316
+--+-+-- +----+-- ++------ ----- 0312,0320 = 0316,0320
+--+-+-- +----+-- -++----- ----- 0312,0322 = 0316,0322
+--+-+-- +----+-- -+-+---- ----- 0312,0325 = 0312,0328 = 0316,0328
+--+-+-- +----+-- -+---+-- ----- 0312,0318 = 0316,0318
+--+-+-- +----+-- -+----+- ----- 0312,0319 = 0316,0319
+--+-+-- +----+-- -+------ +---- 0312,0332 = 0316,0332
+--+-+-- +----+-- -+------ -+--- 0312,0333 = 0316,0333
+--+-+-- +----+-- -+------ --+-- 0312,0334 = 0316,0334
+--+-+-- +----+-- -+------ ---+- 0312,0336 = 0316,0336
+--+-+-- +----+-- -+------ ----+ 0312,0339 = 0316,0339
+--+-+-- +----+-- -+------ ----- 0301,0312 = 0301,0316 = 0312,0316
+--+-+-- +-----+- -+-+---- ----- 0325,0337 = 0328,0337
+--+-+-- +-----+- -+------ ----- 0301,0337 = 0337,0337
+--+-+-- +------+ -+-+---- ----- 0315,0325 = 0315,0328
+--+-+-- +------+ -+------ ----- 0301,0315 = 0315,0315
+--+-+-- +------- ++-+---- ----- 0320,0325 = 0320,0328
+--+-+-- +------- ++------ ----- 0301,0320 = 0320,0320
+--+-+-- +------- -++---- ----- 0322,0325 = 0322,0328
+--+-+-- +------- -++----- ----- 0301,0322 = 0322,0322
+--+-+-- +------- -+-+-+-- ----- 0318,0325 = 0318,0328
+--+-+-- +------- -+-+--+- ----- 0319,0325 = 0319,0328
+--+-+-- +------- -+-+---- +---- 0325,0332 = 0328,0332
+--+-+-- +------- -+-+---- -+--- 0325,0333 = 0328,0333
+--+-+-- +------- -+-+---- --+-- 0325,0334 = 0328,0334
+--+-+-- +------- -+-+---- ---+- 0325,0336 = 0328,0336
+--+-+-- +------- -+-+---- ----+ 0325,0339 = 0328,0339
+--+-+-- +------- -+-+---- ----- 0301,0325 = 0301,0328 = 0325,0328 =
                                  0328,0328
+--+-+-- +------- -+---+-- ----- 0301,0318 = 0318,0318
+--+-+-- +------- -+----+- ----- 0301,0319 = 0319,0319
+--+-+-- +------- -+------ +---- 0301,0332 = 0332,0332
+--+-+-- +------- -+------ -+--- 0301,0333 = 0333,0333
+--+-+-- +------- -+------ --+-- 0301,0334 = 0334,0334
+--+-+-- +------- -+------ ---+- 0301,0336 = 0336,0336
+--+-+-- +------- -+------ ----+ 0301,0339 = 0339,0339
+--+---- ++--+--- -------- -+--- 0324,0326 = 0326,0335
+--+---- ++--+--- -------- ----- 0306,0324 = 0306,0335
+--+---- ++------ -------- -+--- 0306,0326 = 0326,0326
+---++-- +------- -+-+---- ----- 0304,0304 = 0304,0325
-+++---+ -+--+--- -------+ ----- 0302,0324 = 0303,0327
-+++---+ -+------ -+-----+ ----- 0302,0307 = 0303,0329
-++--+-+ +++----- -+----++ ----- 0302,0309 = 0309,0338
-++----+ -++-+--- -------+ ----- 0327,0338 = 0335,0338
-++----+ -++----- -------+ ----- 0302,0338 = 0338,0338
-++----+ -+--+--- -+-----+ ----- 0327,0329 = 0329,0335
-++----+ -+--+--- -------+ ----- 0302,0327 = 0302,0335
-++----- -+--+--- -------+ ----- 0327,0327 = 0327,0335
-+-+---+ -+--+--- -------+ ----- 0303,0324 = 0303,0335
--+--+-- +-+----- -+----+- ----- 0305,0309 = 0309,0309
--+--+-- +------+ -+------ ----- 0305,0314 = 0314,0314
-------- -+--+-+- -------- ----- 0317,0317 = 0317,0335
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SPECIFICITY TABLE

DRB1*03 SSP subtyping

Specificities and sizes of the PCR products of the 29 primer mixes used for DRB1*03 SSP subtyping

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	Amplified DRB1*03 alleles[3]	Other amplified DRB alleles[4]
1	220 bp	**515 bp**	030101-030106, 0304, 0306, 0308, 0310-0313, 0315, 0318-0320, 0322, 0323, 0325, 0326, 0328, 0330-0334, 0336, 0337, 0339	0906, 1159, 1327, 1371
2[5]	85 bp	430 bp	030201-0303, 0327, 0329, 0338	1315, 1319, 1326, 1353, 1357, 1402-140302, 1406, 1412, 1413, 1418-1420, 1424, 1427, 1429, 1440, 1441, 1447-1449, 1451, 1463, 1467
3[6]	190 bp	**515 bp**	030201-030202, 030501-030502, 0309, 0314, 0327, 0329, 0338	1109, 1120, 130201-130203, 130501-130502, 1326, 1329, 1331, 1334, 1336, 1339, 1341, 135002, 1356, 1363, 1365, 1367, 1373, 1374, 1402-140302, 1409, 1413, 1419, 1424, 1427, 1430, 1446-1448, 1451, 1463, 1467, 1608, **DRB3*0108, DRB3*0206, DRB3*0220**
4	190 bp	430 bp	030101-030106, 0303, 0306-0308, 0310-0313, 0315, 0318-0320, 0322-0324, 0326, 0328, 0332-0334, 0336, 0337, 0339	1116, 1140, 1158, 1159, 130101-130103, 1306, 1309, 1310, 1315, 1318, 1320, 1327, 1328, 1332, 1335, 1340, 1342, 1343, 1351,

				1353, 1359, 1361, 1369, 1371, 1377-1380, 1406, 1412, 1417, 1418, 1421, 1429, 1433, 1452, 1459, 1464
5[5]	75 bp	430 bp	0304	
6[5]	105 bp	430 bp	030101-030106, 0304-030502, 0308-0315, 0316[weakly], 0318-0320, 0322, 0323, 0325, 0328, 0330, 0332-0334, 0336, 0337, 0339	1159, 1327, 1341, 1371
7[5]	135 bp	430 bp	0308	1159
8[7]	185 bp	430 bp	030201-0303, 0307, 0321, 0329, 0338	0116, 0422, 1107, 1525, **DRB3*0109, DRB3*0204, DRB3*0219, DRB3*0222, DRB3*0303**
9[5]	135 bp	430 bp	030101-030106, 0304-0306, 0309, 0311, 0313[weakly], 0314-0316, 0318-0320, 0322, 0323, 0325, 0326, 0328, 0330-0334, 0336, 0337, 0339	1327, 1341, 1371, **DRB3*0107**
10[5]	145 bp	430 bp	030201-0303, 0306, 0317, 0326, 0327, 0331, 0335, 0338	0820, 1117, 1137, 1141, 1148, 1150, 1152, 1156, 130301-130302, 130701-1308, 1312, 1313, 1319, 1326, 1332, 1333, 1336-1338, 1340, 1347-1349, 1353, 1355, 1358, 1360, 1365, 1370, 1372, 1376, 1381, 140101-140301, 140501-1409, 1412-1414, 1416, 1418-1420, 1422-1427, 1429, 143201-

				143202, 1434, 1436-1441, 1443-1445, 1447-1449, 1451, 1454-1456, 1458-1460, 1462, 1463, 1467, 1469, 1470, 1474, 1475
11[5,8]	105, 180 bp	**515 bp**	0309, 0313, 0323, 0338	1339, 1354, 1377-1379, 1448, 1464
12[5]	135 bp	430 bp	0310	
13	225 bp	430 bp	0311, 0317, 0324, 0327, 0335	1438
14[5,9]	105, 135 bp	**515 bp**	0312, 0316	
15	155 bp	430 bp	0317, 0337	1139, 1143, 1150, 1459
16	195 bp	430 bp	0314, 0315	0905, 1159, 1327, 1341, 1371
17	255 bp	**515 bp**	0320	110601-110602, 1121, 1147, 1157, 1358, 1381, 1429
18[5]	120 bp	430 bp	030101-030106, 0304-030502, 0307-0310, 0312-0316, 0318-0323, 0325, 0328-0330, 0332-0334, 0336, 0337, 0339	1107, 1525
19[6]	175 bp	**515 bp**	0322	
20[5,10]	75, 120 bp	430 bp	0304[weakly], 0325, 0328, 0331	
21[5]	70 bp	430 bp	0321, 0330	1110, 111201-111202[weakly], 1113, 1205, 1214, 1215, 1357, 1364, 1435, 1465, 1472
22[5]	95 bp	430 bp	0318	
23[5,11]	65, 165 bp	430 bp	0309, 0319, 0323	
24[5,7]	65 bp	430 bp	030201-0303, 0327, 0329, 0338	1315, 1326, 1353, 1402-140302, 1406, 1412, 1413, 1418, 1419, 1424, 1427, 1429, 1446-1448, 1451, 1452, 1463, 1467, **DRB3*0220**

25	180 bp	430 bp	0332	
26[5]	115 bp	**515 bp**	0326, 0333	1144, 1149, 1150
27	230 bp	**515 bp**	0334	
28[12]	160 bp	430 bp	0336	
29	180 bp	430 bp	0339	

[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 DRB1*03SSP 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 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 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 DRB1*03 subtyping.
In addition, wells number 3, 11, 14, 17, 19, 26 and 27 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 within the DR52 group of DRB1 alleles, several DRB1*11, several DRB1*12 as well as many DRB1*13 and DRB1*14 alleles are amplified by some of the DRB1*03 primer mixes. In addition, the DRB1*0116 allele, the DRB1*0422 allele, the DRB1*0820 allele, the DRB1*0905 and 0906 alleles, the DRB1*1525 allele and DRB1*1608 allele are amplified by a few of the DRB1*03 primer mixes. Finally, the DRB3*0107 allele is amplified by primer mix 9, the DRB3*0108 and DRB3*0206 alleles are amplified by primer mix 3, the DRB3*0109, DRB3*0204, DRB3*0219, DRB3*0222 and DRB3*0303 alleles by primer mix 8 and the DRB1*0220 allele by primer mixes 3 and 24.
[5]Specific PCR fragments shorter than 150 base pairs have a lower intensity than longer PCR bands.
[6]Primer mixes 3 and 19 have a tendency of giving rise to primer oligomer formation. Most pronounced for primer mix 19.
[7]The specific primer pairs in primer mixes 8 and 24 yield less product than than the other DRB1*03 primer mixes.
[8]Primer mix 11: Specific PCR fragment of 105 bp in the DRB1*0309 and 0323 alleles. Specific PCR fragment of 180 bp in the DRB1*0313 and 0338 and the 1339, 1354, 1377-1379, 1448 and 1464 alleles.

[9]Primer mix 14: Specific PCR fragment of 105 bp in the DRB1*0316 allele. Specific PCR fragment of 135 bp in the DRB1*0312 allele.
[10]Primer mix 20: Specific PCR fragment of 75 bp in the DRB1*0304[weakly], 0325 and 0331 alleles. Specific PCR fragment of 120 bp in the DRB1*0328 allele.
[11]Primer mix 23: Specific PCR fragment of 65 bp in the DRB1*0309 and 0323 alleles. Specific PCR fragment of 165 bp in the DRB1*0319 allele.
[12]Primer mix 28 may give to nonspecific amplifications.

INTERPRETATION TABLE

DRB1*03 SSP subtyping

Amplification patterns of the DRB1*0301 to *0339 alleles

	Well[5]															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length of spec.	220	85	190	190	75	105	135	185	135	145	105	135	225	105	155	195
PCR product											180			135		
Length of int.	515	430	515	430	430	430	430	430	430	430	515	430	430	515	430	430
pos. control[1]																
5'-primer[2]	26	13	37	37	26	26	26	26	26	13	13	26	13	26	13	26
	5'-g TA3'	5'-g TC3'	5'-Ag A3'	5'-Ag A3'	5'-g TA3'	5'-g TA3'	5'-g TA3'	5'-g TT3'	5'-g TA3'	5'-g TC3'	5'-g TC3'	5'-g TA3'	5'-g TC3'	5'-g TA3'	5'-g TC3'	5'-g TA3'
3'-primer(s)[3]	86	28	86	86	37	47	58	74	57	47	34	57	74	48	51	77
	5'-C CA3'	5'-CT C3'	5'-C AC3'	5'-C CA3'	5'-C gg3'	5'-g gA3'	5'-C CT3'	5'-C CC3'	5'-C AT3'	5'-g gT3'	5'-C CC3'	5'-C Ag3'	5'-C CT3'	5'-CCA3'	5'-CCC3'	5'-A gg3'
											60			57		
											5'-A gg3'			5'-gCT3'		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
DRB1 allele																
*030101-030106	1			4		6			9							
*030201-030202		2	3					8		10						
*0303		2		4				8		10						
*0304	1				5	6			9							
*030501-030502			3			6			9							
*0306	1			4					9	10						
*0307				4				8								
*0308	1			4		6	7									
*0309			3			6			9		11					
*0310	1			4		6						12				
*0311	1			4		6			9				13			
*0312	1			4		6								14		
*0313	1			4		6			w		11					
*0314			3			6			9							16
*0315	1			4		6			9							16
*0316						w			9					14		
*0317										10			13		15	
*0318	1			4		6			9							
*0319	1			4		6			9							
*0320	1			4		6			9							
*0321								8								
*0322	1			4		6			9							
*0323	1			4		6			9		11					
*0324				4									13			
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

INTERPRETATION TABLE

DRB1*03 SSP subtyping

Amplification patterns of the DRB1*0301 to *0339 alleles

Well[5]													
17	18	19	20	21	22	23	24	25	26	27	28	29	
255	120	175	75	70	95	65	65	180	115	230	160	180	Length of spec.
			120			165							PCR product
515	430	515	430	430	430	430	430	430	515	515	430	430	Length of int.
													pos. control[1]
13	47	32	26	37	26	26	28	27	13	13	33	27	5'-primer[2]
5'-g TC3'	5'-g TT3'	5'-TC g3'	5'-g TA3'	5'-g TT3'	5'-g TA3'	5'-g TA3'	5'-gAg3'	5'-AC g3'	5'-g TC3'	5'-g TC3'	5'-AAA3'	5'-C Cg3'	
85	73	77	37	47	45	34	37	73	38	76	73	73	3'-primer(s)[3]
5'-C Ag3'	5'-g gC3'	5'-A gT3'	5'-gTA3'	5'-g gA3'	5'-CCg3'	5'-C CC3'	5'-gTT3'	5'-g gC3'	5'-g Cg3'	5'-gTT3'	5'-g gC3'	5'-g gC3'	
			52			67							
			5'-CTT3'			5'-gAg3'							
17	18	19	20	21	22	23	24	25	26	27	28	29	Well No.
													DRB1 allele
	18												*030101-030106
							24						*030201-030202
							24						*0303
	18		W										*0304
	18												*030501-030502
													*0306
	18												*0307
	18												*0308
	18					23							*0309
	18												*0310
													*0311
	18												*0312
	18												*0313
	18												*0314
	18												*0315
	18												*0316
													*0317
	18				22								*0318
	18					23							*0319
17	18												*0320
	18			21									*0321
	18	19											*0322
	18					23							*0323
													*0324
17	18	19	20	21	22	23	24	25	26	27	28	29	Well No.

Length of spec.	220	85	190	190	75	105	135	185	135	145	105	135	225	105	155	195
PCR product											180			135		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*0325	1					6			9							
*0326	1			4					9	10						
*0327		2	3							10			13			
*0328	1			4		6			9							
*0329		2	3					8								
*0330	1					6			9							
*0331	1								9	10						
*0332	1			4		6			9							
*0333	1			4		6			9							
*0334	1			4		6			9							
*0335										10			13			
*0336	1			4		6			9							
*0337	1			4		6			9						15	
*0338		2	3					8		10	11					
*0339	1			4		6			9							
*0116, 0422								8								
*0820, 1117, 1137, 1141, 1148, 1152, 1156, 130301-130302, 130701-1308, 1312, 1313, 1333, 1337, 1338, 1347-1349, 1355, 1360, 1370, 1372, 1376, 140101-140103, 140501-140503, 140701-1408, 1414, 1416, 1422-142302, 1425, 1426, 143201-143202, 1434, 1436, 1437, 1439, 1443-1445, 1454-1456, 1458, 1460, 1462, 1469, 1470, 1474, 1475										10						
*0905																16
*0906	1															
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

255	120	175	75	70	95	65	65	180	115	230	160	180	Length of spec.
			120			165							PCR product
17	18	19	20	21	22	23	24	25	26	27	28	29	Well No.
	18		20										*0325
									26				*0326
							24						*0327
	18		20										*0328
	18						24						*0329
	18			21									*0330
			20										*0331
	18							25					*0332
	18								26				*0333
	18									27			*0334
													*0335
	18										28		*0336
	18												*0337
							24						*0338
	18											29	*0339
													*0116, 0422
													*0820, 1117, 1137, 1141, 1148, 1152, 1156, 130301-130302, 130701-1308, 1312, 1313, 1333, 1337, 1338, 1347-1349, 1355, 1360, 1370, 1372, 1376, 140101-140103, 140501-140503, 140701-1408, 1414, 1416, 1422-142302, 1425, 1426, 143201-143202, 1434, 1436, 1437, 1439, 1443-1445, 1454-1456, 1458, 1460, 1462, 1469, 1470, 1474, 1475
													*0905
													*0906
17	18	19	20	21	22	23	24	25	26	27	28	29	Well No.

Length of spec.	220	85	190	190	75	105	135	185	135	145	105	135	225	105	155	195
PCR product											180			135		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*110601-110602, 1121, 1147, 1157																
*1107, 1525								8								
*1109, 1120, 130201-130203, 130501-130502, 1329, 1331, 1334, 135002, 1356, 1363, 1367, 1373, 1374, 1430, 1608			3													
*1110, 1113, 1205, 1214, 1215, 1364, 1435, 1465, 1472																
*111201-111202																
*1116, 1140, 1158, 130101-130103, 1306, 1309, 1310, 1318, 1320, 1328, 1335, 1342, 1343, 1351, 1359, 1361, 1369, 1380, 1417, 1421, 1433				4												
*1139, 1143															15	
*1144, 1149																
*1150										10					15	
*1159	1			4		6	7									16
*1315		2		4												
*1319, 1420, 1440, 1441, 1449		2								10						
*1326, 1402, 140301, 1413, 1419, 1424, 1427, 1447, 1451, 1463, 1467		2	3							10						
*1327, 1371	1			4		6			9							16
*1332, 1340				4						10						
*1336, 1365, 1409			3							10						
*1339			3								11					
*1341			3			6			9							16
*1353, 1406, 1412, 1418		2		4						10						
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

255	120	175	75	70	95	65	65	180	115	230	160	180	Length of spec.
			120			165							PCR product
17	18	19	20	21	22	23	24	25	26	27	28	29	Well No.
17													*110601-110602, 1121, 1147, 1157
	18												*1107, 1525
													*1109, 1120, 130201-130203, 130501-130502, 1329, 1331, 1334, 135002, 1356, 1363, 1367, 1373, 1374, 1430, 1608
				21									*1110, 1113, 1205, 1214, 1215, 1364, 1435, 1465, 1472
				w									*111201-111202
													*1116, 1140, 1158, 130101-130103, 1306, 1309, 1310, 1318, 1320, 1328, 1335, 1342, 1343, 1351, 1359, 1361, 1369, 1380, 1417, 1421, 1433
													*1139, 1143
									26				*1144, 1149
									26				*1150
													*1159
							24						*1315
													*1319, 1420, 1440, 1441, 1449
							24						*1326, 1402, 140301, 1413, 1419, 1424, 1427, 1447, 1451, 1463, 1467
													*1327, 1371
													*1332, 1340
													*1336, 1365, 1409
													*1339
													*1341
							24						*1353, 1406, 1412, 1418
17	18	19	20	21	22	23	24	25	26	27	28	29	Well No.

Length of spec.	**220**	**85**	**190**	**190**	**75**	**105**	**135**	**185**	**135**	**145**	**105**	**135**	**225**	**105**	**155**	**195**
PCR product											**180**			**135**		
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
***1354**											**11**					
***1357**		**2**														
***1358, 1381**										**10**						
***1377-1379, 1464**				**4**							**11**					
***140302**		**2**	**3**													
***1429**		**2**		**4**						**10**						
***1438**										**10**			**13**			
***1446**			**3**													
***1448**		**2**	**3**							**10**	**11**					
***1452**				**4**												
***1459**				**4**						**10**					**15**	
DRB1 allele																
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
DRB3*0107									**9**							
DRB3*0108, 0206			**3**													
DRB3*0109, 0204, 0219, 0222, 0303								**8**								
DRB3*0220			**3**													
Well No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**

[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 DRB1*03 subtyping.
In addition, wells number 3, 11, 14, 17, 19, 26 and 27 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.
[2]The codon, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given. Codon numbering as on the 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.
[3]The codon, in the 2nd 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 web site. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.

255	120	175	75	70	95	65	65	180	115	230	160	180	Length of spec.
			120			165							PCR product
17	18	19	20	21	22	23	24	25	26	27	28	29	Well No.
													*1354
				21									*1357
17													*1358, 1381
													*1377-1379, 1464
							24						*140302
17							24						*1429
													*1438
							24						*1446
							24						*1448
							24						*1452
													*1459
													DRB1 allele
17	18	19	20	21	22	23	24	25	26	27	28	29	Well No.
													DRB3*0107
													DRB3*0108, 0206
													DRB3*0109, 0204, 0219, 0222, 0303
							24						DRB3*0220
17	18	19	20	21	22	23	24	25	26	27	28	29	Well No.

[4]Primer mix 11: Specific PCR fragment of 105 bp in the DRB1*0309 and 0323 alleles. Specific PCR fragment of 180 bp in the DRB1*0313 and 0338 and the 1339, 1354, 1377-1379, 1448 and 1464 alleles.
Primer mix 14: Specific PCR fragment of 105 bp in the DRB1*0316 allele. Specific PCR fragment of 135 bp in the DRB1*0312 allele.
[10]Primer mix 20: Specific PCR fragment of 75 bp in the DRB1*0304[weakly], 0325 and 0331 alleles. Specific PCR fragment of 120 bp in the DRB1*0328 allele.
Primer mix 23: Specific PCR fragment of 65 bp in the DRB1*0309 and 0323 alleles. Specific PCR fragment of 165 bp in the DRB1*0319 allele.
"w", may be weakly amplified.

CELL LINE VALIDATION SHEET

DRB1*03 SSP subtyping

					Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.:	200729601	200844102	200729603	200729604	200729605	200729606	200729607	200729608	200729609	200729610	200729611	200729612	200729613	200729614	200729615	200729616
	IHWC cell line		DRB1																	
1	9001	SA	*0101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*1502	*0405	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*1502		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*0301		+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-
5	9009	KAS011	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*0407	*0803	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*0301		+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-
8	9007	DEM	*0401	*1602	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*0402		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*0405		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9067	BTB	*0801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*0802		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*0901		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*1101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9008	WILJON	*1501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*0901	*1101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*1201		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*1302		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*1402		-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-
22	9056	KOSE	*1302	*1401	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
23	9124	IHL	*0803	*1414	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
24	9035	JBUSH	*1101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*0301		+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-
27	9191	CH1007	*0405	*1001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*0416	*0701	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*0302		-	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-
31	9019	DUCAF	*0301		+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-
32	9297	HAG	*1303		-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
33	9098	MT14B	*0404		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*1101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*0403		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*0403	*0406	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*1301		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*1402		-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-
39	9315	CML	*0301	*0401	+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-
40	9134	WHONP199	*0701	*0901	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*1302		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*0803		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*0901		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*1401		-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
45	9239	SHJO	*0701		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*1501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*1104	*1201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*0103		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CELL LINE VALIDATION SHEET

DRB1*03 SSP subtyping

					Well												
					17	18	19	20	21	22	23	24	25	26	27	28	29
				Prod. No.:	200729617	200844118	200844119	200729620	200729621	200729622	200729623	200729624	200844125	200844126	200844127	200844128	200844129
	IHWC cell line		DRB1														
1	9001	SA	*0101		-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*1502	*0405	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*1502		-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*0301		-	+	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*0407	*0803	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*0301		-	+	-	-	-	-	-	-	-	-	-	-	-
8	9007	DEM	*0401	*1602	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*0402		-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*0405		-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-
13	9067	BTB	*0801		-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*0802		-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*0901		-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*1101		-	-	-	-	-	-	-	-	-	-	-	-	-
17	9008	WILJON	*1501		-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*0901	*1101	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*1201		-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*1302		-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*1402		-	-	-	-	-	-	-	+	-	-	-	-	-
22	9056	KOSE	*1302	*1401	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*0803	*1414	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*1101		-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*0301		-	+	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*0405	*1001	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*0416	*0701	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*0302		-	-	-	-	-	-	-	+	-	-	-	-	-
31	9019	DUCAF	*0301		-	+	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*1303		-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*0404		-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*1101		-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*0403		-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*0403	*0406	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*1301		-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*1402		-	-	-	-	-	-	-	+	-	-	-	-	-
39	9315	CML	*0301	*0401	-	+	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*0701	*0901	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*1302		-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*0803		-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*0901		-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*1401		-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*1501		-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*1104	*1201	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*0103		-	-	-	-	-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® DRB1*03 SSP

Product number: **101.113-24/04 – including *Taq* pol.**
101.113-24u/04u – without *Taq* pol.
Lot number: **36E**
Expiry date: **2010-March-01**
Number of tests: **24 tests – Product No. 101.113-24**
4 tests – Product No. 101.113-04
Number of wells per test: **29**

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2007-296-01	13	2007-296-13	25	2008-441-25
2	2008-441-02	14	2007-296-14	26	2008-441-26
3	2007-296-03	15	2007-296-15	27	2008-441-27
4	2007-296-04	16	2007-296-16	28	2008-441-28
5	2007-296-05	17	2007-296-17	29	2008-441-29
6	2007-296-06	18	2008-441-18		
7	2007-296-07	19	2008-441-19		
8	2007-296-08	20	2007-296-20		
9	2007-296-09	21	2007-296-21		
10	2007-296-10	22	2007-296-22		
11	2007-296-11	23	2007-296-23		
12	2007-296-12	24	2007-296-24		

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

No DNAs carrying the alleles to be amplified by primer solutions 5, 7, 11, 12, 13, 14, 15, 16, 17, 19, 20, 21, 22, 23, 25, 26, 27, 28 and 29 were available. The specificities of the primers in primer solutions 5, 7, 11, 12, 13, 14, 15, 16, 17, 21 and 26 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 11 and 14 one of the 3'-primer were not possible to test. In primer solution 21 one of the 5'-primers was not possible to test. In primer solutions 19, 25, 28 and 29 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 20, 22, 23 and 27 it was only possible to test the 5'-primers, the 3'-primers were not possible to test.

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

Date of approval: 2008-April-01

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*03
Product number: 101.113-24/04, 101.113-24u/04u
Lot number: 36E
Intended use: DRB1*03 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
Hasselstigen 1
SE-133 33 Saltsjöbaden, Sweden
Phone: +46-8-717 88 27
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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 9001:2000 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
2008-April-01

Olle Olerup
Managing Director

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