Olerup SSP® DR low resolution

Product number:	**101.101-48/12 – including *Taq* pol.**
Lot number:	**83F**
Expiry date:	**2011-April-01**
Number of tests:	**48 tests – Product No. 101.101-48**
	12 tests – Product No. 101.101-12
Number of wells per test:	**23 + 1**
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. 83F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® DR LOW RESOLUTION LOT

The DR low resolution specificity and interpretation tables have been updated for the HLA-DRB1 alleles described since the previous *Olerup* SSP® DR low resolution lot was made **(Lot No. 40E)**.

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

Well	5'-primer	3'-primer	rationale
8	Modified	Modified	Increased yield of specific PCR product.
23	-	Exchange	Primer exchanged to avoid co-amplification of the DRB1*0905 allele.

Well **24** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup* SSP® HLA Class I, DRB, DQB1 and DPB1 amplicons as well as the amplicons generated by control primer pairs.

PCR product sizes range from 75 to 430 base pairs.
The PCR product generated by the control primer pair is 430 base pairs.

Length of PCR product	**105**	**200**	**105**	**80**	**75**	**80**
5'-primer[1]	**164**	**340**	**440**	**45**	**45**	**43**
	5'-CAC3'	**5'-Agg3'**	**5'-TTA3'**	**5'-Tg g3'**	**5'-Tg g3'**	**5'-Tg g3'**
3'-primer[2]	**231**	**2nd I**	**507**	**59**	**58**	**57**
	5'-TgC3'	**5'-AAA3'**	**5'-TTg3'**	**5'-CTC3'**	**5'-ggC3'**	**5'-CTC3'**
A*	**+**	**+**	**+**			
B*	**+**	**+**	**+**			
Cw*	**+**	**+**	**+**			
DRB1				**+**	**+**	
DRB3				**+**	**+**	
DRB5				**+**		
DQB1					**+**	
DPB1						**+**

[1]The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and codonnumbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

[2]The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and 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.

PRODUCT DESCRIPTION

DR low resolution

CONTENT

The primer set contains 5'- and 3'-primers for grouping the DRB1*0101 to DRB1*1003 alleles into the corresponding serological groups DR1 to DR18 as well as primer pairs for recognizing the DRB3, DRB4 and DRB5 groups of 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**

Wells 1 to 23 – DR low resolution primers.
Well 24 – Negative Control.
The 24 well cut PCR plate is marked with 'DR low' in silver/gray ink.
Well No. 1 is marked with the Lot No.'83F'.
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 HLA-DRB alleles will be amplified by the 23 wells of the DR low resolution primer set, **wells 1 to 23**. Thus, the interpretation of DR low resolution typings is not influenced by other HLA class II genes.

UNIQUELY IDENTIFIED ALLELES

All the HLA-DRB1, -DRB3, -DRB4[1] and –DRB5 alleles, i.e. **DRB1*010101 to 1003, DRB3*010101 to DRB3*0303, DRB4*01010101 to DRB4*0107, DRB5*010101 to DRB5*0205**, recognized by the HLA Nomenclature Committee in April 2009[2] will be amplified by the primers in the DR low resolution SSP kit. The HLA-DRB alleles will be grouped into their corresponding serological specificities[3].

[1]The DRB4*0201N and DRB4*0301N null alleles will not be amplified by the DR low resolution primer set.
[2]DRB alleles listed on the IMGT/HLA web page 2008-April-17, release 2.25.0, www.ebi.ac.uk/imgt/hla.
[3]The DRB1*0809, DRB1*0821 and DRB1*1415 alleles yield identical amplification patterns except for the specific PCR product yielded by the DRB3 gene in linkage disequilibrium with the DRB1*1415 allele.
The DRB1*0820, DRB1*1318, DRB1*1347 and DRB1*1355 alleles yield identical amplification patterns except for the specific PCR product yielded by the DRB3 gene in linkage disequilibrium with the DRB1*1318, DRB1*1347 and DRB1*1355 alleles.
The DRB1*0831 and DRB1*1167 alleles yield identical amplification patterns except for the specific PCR product yielded by the DRB3 gene in linkage disequilibrium with the DRB1*1167 allele.

SPECIFICITY TABLE

DR low resolution primer set

Specificities and sizes of the PCR products of the 23 primer mixes of the DR low resolution primer set

Primer Mix	Size of spec. PCR product[1]	Size of control band[2]	DR serology[3]	Amplified HLA-DRB[4] alleles
1	205 bp	**515 bp**	1	010101-010205, 0104-0121
2	195 bp	430 bp	103	0103
3	215, 260 bp	430 bp	15	15010101-1532
4	210 bp	430 bp	16	160101-160502, 1607-1613N
5	220 bp	430 bp	3, 17, 18, 11	03010101-0341, 1107, 1153
6[5,6]	80, 210 bp	430 bp	3, 17, 11, 13, 14	03010101-030106, 0304-0306, 0308-0316, 0318-0320, 0322, 0323, 0325, 0326, 0328, 0330, 0331, 0333, 0334, 0336, 0337, 110201-1103, 111101-111102, 111401-111402, 1116, 1120, 1121, 1136, 1140, 1141, 1148, 1159, 1163, 116501-116502, 1168, 1170, 130101-1304, 1308, 1310, 1315-1317, 1319, 1320, 1322-1324, 1327-1329, 1331-1341, 1343, 1345, 1348, 1351-1354, 1357, 1359, 1361, 1363-1366, 1368-1376, 1378-1381, 1383-1385, 1387, 1416, 1419, 1421, 1482
7[5,6]	80, 210 bp	430 bp	3, 18, 11, 13, 14	030201-0303, 0327, 0329, 0338, 111301-111302[weakly], 1126, 1134, 1315, 1319, 1326, 1344, 1353, 1357, 1385, 1386, 1402-140302, 1406, 1409, 1412, 1413, 1417-1421, 1424, 1427, 1429, 1430, 143201-143202[weakly], 1433, 1440, 1441, 1447-1449, 1451, 1463, 1465[weakly], 1467, 1477, 1478, 1480, 1481, 1483
8[5,6]	100, 175 bp	430 bp	4	040101-0478
9	205, 230	430 bp	7,	07010101-070103, 0703-0717,

	bp		13, 14	1317, 1450
10	165, 215, 250 bp	**515 bp**	8, 11, 12, 14	080101-080203, 080302-0819, 0821-0836, 1167, 1204, 1216, 1411, 1415, 1468
11[5]	85, 130, 180 bp	430 bp	3, 9, 11	0308, 090102-0908, 1107, 1153
12	205 bp	430 bp	10	100101-1003
13[5,8]	100, 170 bp	430 bp	11, 3, 8	0308, 0831, 110101-1170, 1172
14[5]	85, 105 bp	430 bp	12, 8	0832, 120101-120204, 120302-1219
15	210 bp	430 bp	13, 8, 11, 14	0820, 110101-110404, 110601-110602, 110801-111202, 111401-1116, 1118-1121, 1123-1125, 112701-1133, 1135-1151, 115401-115402, 1156-1166, 1168, 1170, 1172, 130101-1308, 1310-1316, 1318-1343, 1345-1385, 1387, 1388 140301-140302, 1412, 1416, 1419, 1421, 1422, 1425, 1427, 1440, 1453, 1463, 1467, 1469, 1474, 1477, 1478, 1484, 1485
16[6]	195, 210 bp	430 bp	13, 8, 11, 12, 14	080101-080203, 080401-0809, 0811, 0816, 0817, 0820-0822, 0824, 0826, 0828, 0831, 110101-110602, 1109-111202, 111401-1116, 1120, 1121, 1123-1125, 112701-1130, 1132, 1133, 1135-1141, 1143, 1144, 1146-1151, 115401-1156, 1158-1163, 116501-1170, 1172, 120201-120204, 1213, 1215, 1216, 1218, 1219 130101-130201, 130203, 1304-130502, 130701-1309, 131101-131102, 131401-1324, 1326-1329, 1331, 1332, 1334-1336, 1338-1343, 1345-1355, 1357, 1359, 1361-1365, 1367-1376, 1378-1380, 1383, 1384, 1387 1415, 1416, 1422, 1424, 1425, 1427, 1437, 1453, 1473

17	175 bp	430 bp	3, 8, 13, 14	03010101-0307, 0309, 0311-0341, 0820, 130101-1316, 1318-1342, 1344, 1346-1366, 1368-1388, 1402-140302, 140501-1406, 1409, 1412-1414, 1417-1421, 142301, 1424, 1427, 1429, 1430, 1433, 1436, 1437, 1440-1445, 1447, 1448, 1451, 1456, 1459, 1463-1465, 1467, 1477, 1478, 1480, 1481, 1483-1485
18[5-7]	100, 140 bp	430 bp	14, 4, 8,13	0462, 0469, 0473, 0808, 1169, 1345, 140101-140102, 1404, 140701-140702, 1410, 1416, 1422, 1425, 1426, 1428, 1431-143202, 1435, 1437-1439, 1449, 1450, 1453-1455, 1457, 1458, 1460-1462, 1468-1471, 1473-1476, 1479, 1482
19[5-9]	110, 135, 170 bp	**515 bp**	14, 3, 9, 11, 12, 13, 15	0308, 0310, 090102-090202, 0904-0908, 110404, 1109-111002, 111202-111302, 1116, 1117, 1120, 1122, 1140, 1146, 1152, 1158, 1159, 1204, 1343, 140101-1402, 1404-1411, 1413, 1414, 1416-142302, 1426, 1428-1436, 1438, 1439, 1441, 1443-1452, 1454-1457, 1459-1462, 1464, 1465, 1468, 1470-1476, 1479-1483 1527
20[5-7]	110, 175, 225 bp	430 bp	14, 3, 8, 11, 13, 15, 16	0310, 0809, 0820, 0821, 0832, 0835, 111301-111302, 1117, 1123, 1125, 1131, 1145, 1152, 1155, 1164, 1313, 1318, 1343, 1345, 1347, 1355, 140101-140103, 140301-140503, 140701-1408, 1410-1412, 1414-1416, 1418, 1422-142302, 1425-1428, 1431-143202, 1434-1436, 1438-1440, 1442-1445, 1449, 1450, 1453-1465, 1467-1479, 1481, 1482, 1484, 1485 1521[weakly], 1604[weakly]
21	230 bp	430 bp	52	DRB3*01010201-0113, 0201-0224,

				030101-0303
22[10]	215 bp	430 bp	53	DRB4*01010101-0107
23	175 bp	430 bp	51	DRB5*010101-0113, 0202-0205

[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 DR low resolution SSP subtypings.
When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective 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, e.g. the primers in wells 3, 18, 19 and 20.
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 DR low resolution typing.
In addition, wells number 10 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]The serological reactivity of all DRB alleles is not known. In this table we use the information in the HLA Dictionary 2004 on the www.ebi.ac.uk/imgt/hla web site and the information available at the www.worldmarrow.org web site and have also inferred the serological grouping from the naming of the sequence-defined allele.
[4]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 set 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.
The DRB1*0809, DRB1*0821 and DRB1*1415 alleles yield identical amplification patterns except for the specific PCR product yielded by the DRB3 gene in linkage disequilibrium with the DRB1*1415 allele.
The DRB1*0820, DRB1*1318, DRB1*1347 and DRB1*1355 alleles yield identical amplification patterns except for the specific PCR product yielded by the DRB3 gene in linkage disequilibrium with the DRB1*1318, DRB1*1347 and DRB1*1355 alleles.
The DRB1*0831 and DRB1*1167 alleles yield identical amplification patterns except for the specific PCR product yielded by the DRB3 gene in linkage disequilibrium with the DRB1*1167 allele.
[5]Specific PCR fragments shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR bands.
[6]Individual alleles can give to rise to two differently sized specific PCR fragments in these primer mixes.
[7]These primer mixes may give rise to nonspecific amplifications.
[8]Primer mix 13 may give rise a primer oligomer formation.
[9]Primer mix 19 has a tendency of primer oligomer formation and also has an intense primer cloud due to the high number of primers present in the primer mix.
[10]The DRB4*01030102N allele is amplified by the primer pair in well No. 22, whereas the DRB4*0201N and DRB4*0301N null alleles are not amplified by this primer pair.

INTERPRETATION TABLE

DR low resolution SSP typing

Amplification patterns of the DRB1*0101 to DRB1*1003 alleles

		Well											
		1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.		205	195	215	210	220	80	80	100	205	165	85	205
PCR product(s)				260			210	210	175	230	215	130	
											250	180	
Length of int. pos ctr[1]		515	430	430	430	430	430	430	430	430	515	430	430
5'-primer(s)[2]		14	14	13	13	13	13	13	13	14	16	26	31
		5'-gAA3'	5'-gAA3'	5'-Agg3'	5'-Agg3'	5'-g TC3'	5'-g TC3'	5'-g TC3'	5'-A CA3'	5'-AT A3'	5'-gT T3'	5'-TAT3'	5'-gC g3'
					13		16		13	14	16	58	
					5'-AAg3'		5'-gT T3'		5'-A CC3'	5'-AT A3'	5'-gT T3'	5'-gAg3'	
									13	16			
									5'-A TA3'	5'-gT T3'			
3'-primer(s)[3]		67	67	70	67	73	26	28	33	71	58	57	86
		5'-gAg3'	5'-gAT3'	5'-CTg3'	5'-gAA3'	5'-g gC3'	5'-g gT3'	5'-CT C3'	5'-gTg3'	5'-CTC3'	5'-C CT3'	5'-C gA3'	5'-C AC3'
		71		71	67	73	71	70	58	73	74	73	86
		5'-gC g3'		5'-CgC3'	5'-gAg3'	5'-g gC3'	5'-g CT3'	5'-CTg3'	5'-C gg3'	5'-g gC3'	5'-CAg3'	5'-g gC3'	5'-C CA3'
				86	72					77	86	78	
				5'-C CA3'	5'-gC g3'					5'-A AT3'	5'-C AC3'	5'-CAC3'	
										78			
										5'-CAC3'			
Well No.	**DR**	1	2	3	4	5	6	7	8	9	10	11	12
DRB1 allele[4]	**ser[5]**												
***010101-010205, 0104-0121**	**1**	**1**											
***0103**	**103**		**2**										
***03010101-030106, 0304-0306, 0309, 0311-0316, 0318-0320, 0322, 0323, 0325, 0326, 0328, 0330, 0331, 0333, 0334, 0336, 0337**	**17**					**5**	**6**						
***030201-0303, 0327, 0329, 0338**	**18**					**5**		**7**					
***0307, 0317, 0321, 0324, 0332, 0335, 0339-0341**	**-**					**5**							
***0308**	**-**					**5**	**6**					**11**	
***0310**	**-**					**5**	**6**						
Well No.		1	2	3	4	5	6	7	8	9	10	11	12

INTERPRETATION TABLE

DR low resolution SSP typing

Amplification patterns of the DRB1*0101 to DRB1*1003 alleles

Well												
13	14	15	16	17	18	19	20	21	22	23		
100	85	210	195	175	100	110	110	230	215	175		Length of spec.
170	105		210		140	135	175					PCR product(s)
						170	225					
430	430	430	430	430	430	515	430	430	430	430		Length of int. pos ctr[1]
13	16	10	10	13	37	26	13	10	28	13		5'-primer(s)[2]
5'-g TC3'	5'-gT T3'	5'-g CT3'	5'-g CT3'	5'-g TC3'	5'-g TA3'	5'-g TA3'	5'-g TC3'	5'-g CT3'	5'-g AT3'	5'-g TA3'		
16		13	13		37	34	34	10		13		
5'-gT C3'		5'-g TC3'	5'-g TC3'		5'-g TT3'	5'-CAg3'	5'-CAg3'	5'-g CT3'		5'-g TA3'		
38			16									
5'-C gT3'			5'-gT T3'									
			16									
			5'-gT C3'									
58	30	70	67	58	57	57	57	73	87	57		3'-primer(s)[3]
5'-C CT3'	5'-gTg3'	5'-gTC3'	5'-gAA3'	5'-C gg3'	5'-C Ag3'	5'-C Ag3'	5'-C Ag3'	5'-g gC3'	5'-CTC3'	5'-gC g3'		
58	38	71	67	58	71	58	60	73		58		
5'-C CT3'	5'-CAg3'	5'-g CT3'	5'-gAA3'	5'-C Ag3'	5'-CgC3'	5'-C CT3'	5'-gTg3'	5'-g gC3'		5'-C CT3'		
			71			70	70					
			5'-CTC3'			5'-CTg3'	5'-T CC3'					
			71			70	74					
			5'-CgC3'			5'-T CC3'	5'-CAg3'					
13	14	15	16	17	18	19	20	21	22	23	DR	Well No.
											ser[5]	DRB1 allele[4]
											1	*010101-010205, 0104-0121
											103	*0103
				17							17	*03010101-030106, 0304-0306, 0309, 0311-0316, 0318-0320, 0322, 0323, 0325, 0326, 0328, 0330, 0331, 0333, 0334, 0336, 0337
				17							18	*030201-0303, 0327, 0329, 0338
				17							-	*0307, 0317, 0321, 0324, 0332, 0335, 0339-0341
13						19					-	*0308
						19	20				-	*0310
13	14	15	16	17	18	19	20	21	22	23		Well No.

Well No.		1	2	3	4	5	6	7	8	9	10	11	12
*040101-0461, 0463-0468, 0470-0472, 0474-0478	4								8				
*0462, 0469, 0473	-								8				
*07010101-070103, 0703-0717	7									9			
*080101-080203, 080401-0807, 0811, 0816, 0817, 0822, 0824, 0826, 0828	8										10		
*080302, 0810, 0812-0815, 0818, 0819, 0823, 0825, 0827, 0829, 0830, 0833, 0834, 0836	8										10		
*0808	-										10		
*0809, 0821, 1415	8										10		
*0820, 1318, 1347, 1355	8,13												
*0831, 1167	-										10		
*0832	-										10		
*0835	-										10		
*090102-090202, 0904-0908	9											11	
*0903	9											11	
*100101-1003	10												12
*110101-110107, 110401-110403, 110601-110602, 111201, 1115, 1124, 112701-1130, 1132, 1133, 1135, 1137-1139, 1143, 1144, 1147, 1149-1151, 115401-115402, 1156, 1160-1162, 1166, 1172	11												
*110201-1103, 111101-111102, 111401-111402, 1121, 1136, 1141, 1148, 1163, 116501-116502, 1168, 1170	11						6						
*110404, 1109-111002, 111202, 1146, 1158	11												
*1105	11												
*1107, 1153	-					5						11	
*110801-110802, 1118-111902, 1142, 1157	11												
*111301-111302	11							w					
Well No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

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13	14	15	16	17	18	19	20	21	22	23		Well No.
											4	*040101-0461, 0463-0468, 0470-0472, 0474-0478
					18						-	*0462, 0469, 0473
											7	*07010101-070103, 0703-0717
			16								8	*080101-080203, 080401-0807, 0811, 0816, 0817, 0822, 0824, 0826, 0828
											8	*080302, 0810, 0812-0815, 0818, 0819, 0823, 0825, 0827, 0829, 0830, 0833, 0834, 0836
			16		18						-	*0808
			16				20				8	*0809, 0821, 1415
		15	16	17			20				8,13	*0820, 1318, 1347, 1355
13			16								-	*0831, 1167
	14						20				-	*0832
							20				-	*0835
						19					9	*090102-090202, 0904-0908
											9	*0903
											10	*100101-1003
13		15	16								11	*110101-110107, 110401-110403, 110601-110602, 111201, 1115, 1124, 112701-1130, 1132, 1133, 1135, 1137-1139, 1143, 1144, 1147, 1149-1151, 115401-115402, 1156, 1160-1162, 1166, 1172
13		15	16								11	*110201-1103, 111101-111102, 111401-111402, 1121, 1136, 1141, 1148, 1163, 116501-116502, 1168, 1170
13		15	16			19					11	*110404, 1109-111002, 111202, 1146, 1158
13			16								11	*1105
13											-	*1107, 1153
13		15									11	*110801-110802, 1118-111902, 1142, 1157
13						19	20				11	*111301-111302
13	14	15	16	17	18	19	20	21	22	23		Well No.

Well No.		1	2	3	4	5	6	7	8	9	10	11	12
*1116, 1120, 1140, 1159	-						6						
*1117, 1152	11												
*1122	-												
*1123, 1125	11												
*1126, 1134	11							7					
*1131, 1145, 1164	-												
*1155	-												
*1169	-												
*120101-120102, 120302, 1205-1212, 1214, 1217	12												
*120201-120204, 1213, 1215, 1218, 1219	12												
*1204	-										10		
*1216	-										10		
*130101-130201, 130203, 1304, 1308, 1316, 1320, 1322-1324, 1327-1329, 1331, 1332, 1334-1336, 1338-1341, 1348, 1351, 1352, 1354, 1359, 1361, 1363-1365, 1368-1376, 1378-1380, 1383, 1384, 1387	13						6						
*130202, 130301-130302, 1310, 1333, 1337, 1366, 1381	13						6						
*130501-130502, 130701-130702, 131101-131102, 131401-131403, 1321, 1342, 1346, 1349-135002, 1362	13												
*1306, 1312, 1325, 1330, 1356, 1358, 1360, 1377, 1382, 1388	13												
*1309	13												
*1313	-												
*1315, 1319, 1353, 1357	-						6	7					
*1317	13						6			9			
*1326	-							7					
*1343	-						6						
*1344, 1386	-							7					
*1345	-						6						
Well No.		1	2	3	4	5	6	7	8	9	10	11	12

13	14	15	16	17	18	19	20	21	22	23		Well No.
13		15	16			19					-	*1116, 1120, 1140, 1159
13						19	20				11	*1117, 1152
13						19					-	*1122
13		15	16				20				11	*1123, 1125
13											11	*1126, 1134
13		15					20				-	*1131, 1145, 1164
13			16				20				-	*1155
13			16		18						-	*1169
	14										12	*120101-120102, 120302, 1205-1212, 1214, 1217
	14		16								12	*120201-120204, 1213, 1215, 1218, 1219
	14					19					-	*1204
	14		16								-	*1216
		15	16	17							13	*130101-130201, 130203, 1304, 1308, 1316, 1320, 1322-1324, 1327-1329, 1331, 1332, 1334-1336, 1338-1341, 1348, 1351, 1352, 1354, 1359, 1361, 1363-1365, 1368-1376, 1378-1380, 1383, 1384, 1387
		15		17							13	*130202, 130301-130302, 1310, 1333, 1337, 1366, 1381
		15	16	17							13	*130501-130502, 130701-130702, 131101-131102, 131401-131403, 1321, 1342, 1346, 1349-135002, 1362
		15		17							13	*1306, 1312, 1325, 1330, 1356, 1358, 1360, 1377, 1382, 1388
			16	17							13	*1309
		15		17			20				-	*1313
		15	16	17							-	*1315, 1319, 1353, 1357
			16								13	*1317
		15	16	17							-	*1326
		15	16			19	20				-	*1343
				17							-	*1344, 1386
		15	16		18		20				-	*1345
13	14	15	16	17	18	19	20	21	22	23		Well No.

Well No.		1	2	3	4	5	6	7	8	9	10	11	12
*1367	-												
*1385	-						6	7					
*140101-140102, 1404, 140701-140702, 1410, 1426, 1428, 1431, 1435, 1438, 1439, 1454, 1455, 1457, 1460-1462, 1470, 1471, 1475, 1476, 1479	14												
*140103, 1408, 142302, 1434, 1472	-												
*1402, 1406, 1409, 1413, 1417, 1420, 1429, 1430, 1433, 1441, 1447, 1448, 1451, 1480, 1483	14							7					
*140301-140302, 1412, 1440, 1463, 1467, 1477, 1478	14							7					
*140501-140503, 1414, 142301, 1436, 1443-1445, 1456, 1459, 1464	14												
*1411	-										10		
*1416	6						6						
*1418, 1481	-							7					
*1419, 1421	-						6	7					
*1422	-												
*1424	-							7					
*1425, 1453	-												
*1427	14							7					
*143201-143202	-							w					
*1437	-												
*1442													
*1446, 1452	-												
*1449	-							7					
*1450	-									9			
*1458	-												
*1465	-							w					
*1468	-										10		
*1469, 1484, 1485	-												
*1473	-												
*1474	-												
*1482	-						6						
Well No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

13	14	15	16	17	18	19	20	21	22	23		Well No.
		15	16								-	*1367
		15		17							-	*1385
					18	19	20				14	*140101-140102, 1404, 140701-140702, 1410, 1426, 1428, 1431, 1435, 1438, 1439, 1454, 1455, 1457, 1460-1462, 1470, 1471, 1475, 1476, 1479
						19	20				-	*140103, 1408, 142302, 1434, 1472
				17		19					14	*1402, 1406, 1409, 1413, 1417, 1420, 1429, 1430, 1433, 1441, 1447, 1448, 1451, 1480, 1483
		15		17			20				14	*140301-140302, 1412, 1440, 1463, 1467, 1477, 1478
				17		19	20				14	*140501-140503, 1414, 142301, 1436, 1443-1445, 1456, 1459, 1464
						19	20				-	*1411
		15	16		18	19	20				6	*1416
				17		19	20				-	*1418, 1481
		15		17		19					-	*1419, 1421
		15	16		18	19	20				-	*1422
			16	17							-	*1424
		15	16		18		20				-	*1425, 1453
		15	16	17			20				14	*1427
					18	19	20				-	*143201-143202
			16	17	18						-	*1437
				17			20					*1442
						19					-	*1446, 1452
					18	19	20				-	*1449
					18	19	20				-	*1450
					18		20				-	*1458
				17		26	27				-	*1465
					18	19	20				-	*1468
		15			18		20				-	*1469, 1484, 1485
			16		18	19	20				-	*1473
		15			18	19	20				-	*1474
					18	19	20				-	*1482
13	14	15	16	17	18	19	20	21	22	23		Well No.

Well No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
***15010101-1520, 1522-1526, 1528-1532**	**15**			**3**									
***1521**	**-**			**3**									
***1527**	**-**			**3**									
***160101-1603, 160501-160502, 1607-1613N**	**16**				**4**								
***1604**	**16**				**4**								
Well No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
DRB1 allele[4]	**ser[5]**												
DRB3*01010201-0113, 0201-0224, 030101-0303	**52**												
DRB4*01010101-0107	**53**												
DRB5*010101-0113, 0202-0205	**51**												
Well No.	**ser[5]**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

[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 DR low resolution typing.
In addition, wells number 10 and 19 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.

13	14	15	16	17	18	19	20	21	22	23		Well No.
											15	*15010101-1520, 1522-1526, 1528-1532
							w				-	*1521
						19					-	*1527
											16	*160101-1603, 160501-160502, 1607-1613N
							w				16	*1604
13	14	15	16	17	18	19	20	21	22	23		Well No.
											ser[5]	DRB1 allele[4]
								21			52	DRB3*01010201-0113, 0201-0224, 030101-0303
									22		53	DRB4*01010101-0107
										23	51	DRB5*010101-0113, 0202 0205
13	14	15	16	17	18	19	20	21	22	23	ser[5]	Well No.

[4]The sequence of the DRB1*0702 allele has been shown to be identical to DRB1*070101.
The sequence of the DRB1*080301 allele has been shown to be identical to DRB1*080302.
The sequence of the DRB1*090101 allele has been shown to be identical to DRB1*090102.
The sequence of the DRB1*120301 allele has been shown to be identical to DRB1*1201.
The sequence of the DRB1*1606 allele has been shown to be identical to DRB1*1605.
The DRB4*0101102N allele has been renamed DRB4*0103102N.
The sequence of the DRB5*0201 allele has been shown to be identical to DRB5*0202.
The DRB1*0809, DRB1*0821 and DRB1*1415 alleles yield identical amplification patterns except for the specific PCR product yielded by the DRB3 gene in linkage disequilibrium with the DRB1*1415 allele.
The DRB1*0820, DRB1*1318, DRB1*1347 and DRB1*1355 alleles yield identical amplification patterns except for the specific PCR product yielded by the DRB3 gene in linkage disequilibrium with the DRB1*1318, DRB1*1347 and DRB1*1355 alleles.
The DRB1*0831 and DRB1*1167 alleles yield identical amplification patterns except for the specific PCR product yielded by the DRB3 gene in linkage disequilibrium with the DRB1*1167 allele.
[5]The serological reactivity of all DRB alleles is not known. In this table we use the information in the HLA Dictionary 2004 on the www.ebi.ac.uk/imgt/hla web site and the information available at the www.worldmarrow.org web site and have also inferred the serological grouping from the naming of the sequence-defined allele.
'ser', serological HLA specificity.
'w', may be weakly amplified.

CELL LINE VALIDATION SHEET

DR low resolution primer set

					Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.:	200850201	200850202	200850203	200850204	200850205	200850206	200850207	200959108	200850209	200850210	200850211	200850212	200850213	200850214	200850215	200850216
	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

DR low resolution primer set

					Well						
					17	18	19	20	21	22	23
				Prod. No.:	200850217	200850218	200850219	200850220	200850221	200850222	200957023
	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® DR low resolution

Product number: **101.101-48/12 – including *Taq* pol.**
Lot number: **83F**
Expiry date: **2011-April-01**
Number of tests: **48 tests – Product No. 101.101-48**
12 tests – Product No. 101.101-12
Number of wells per test: **23 + 1**

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2008-502-01	9	2008-502-09	17	2008-502-17
2	2008-502-02	10	2008-502-10	18	2008-502-18
3	2008-502-03	11	2008-502-11	19	2008-502-19
4	2008-502-04	12	2008-502-12	20	2008-502-20
5	2008-502-05	13	2008-502-13	21	2008-502-21
6	2008-502-06	14	2008-502-14	22	2008-502-22
7	2008-502-07	15	2008-502-15	23	2009-570-23
8	2009-591-08	16	2008-502-16		

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

The reactivities of one additional 3'-primer in primer solution 3, one additional 3'-primer in primer solution 4, one additional 5'-primer in primer solution 6, one additional 3'-primer in primer solution 9, one additional primer pair in primer solutions 8, 9 and 11, two additional 5'-primers in primer solution 13, one additional primer pair in primer solution 18, one additional 3'-primer in primer solution 19 and one additional 3'-primer in primer solution 20 were tested by separately adding another 5'-primer or 3'-primer. One of the 5'-primers in primer solution 10 and one of the 5'-primers in primer solution 13 were not possible to test.

The negative control primer pairs, **Production No. 2008-417-01**, can detect contamination with PCR products diluted 10^{-7}.

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

Date of approval: 2009-May-30

Approved by:

Quality Control Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DR low resolution
Product number: 101.101-48/12
Lot number: 83F

Intended use: DRB1 low 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 9001:2000 and ISO 13485:2004, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, 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-30

Olle Olerup
Managing Director

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