

Olerup SSP® DR low resolution

Product number:	101.101-48/12 – including <i>Taq</i> pol.
Lot number:	76G
Expiry date:	2012-February-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. 76G.

**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. 83F**).

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	New primers for the DRB1*0123 and DRB1*0124 alleles.
3	-	Added, exchanged	Primers exchanged and added to avoid co-amplification of DRB1*16 alleles.
5	Added	-	Primer added for the DRB1*0342 allele.
18	Added	Added	Primer pair added for the DRB4*01030102N allele.
19	-	Removed	One 3'-primer removed and exchanged positive control primer pair, to decrease unspecific amplifications.
21	Added	Exchanged	5'-primer added for the DRB3*0114 allele, exchanged 3-primer for improved allelic resolution.
22	-	Added	Primer pair added for the DRB4*0108 allele.

Change in revision R01 compared to R00:

1. The DRB1*1419 and 1421 alleles are weakly amplified in primer mix 19.

Length of PCR product	105	200	105	80	75	80
5'-primer¹	164	340	440	45	45	43
	5'-CAC ^{3'}	5'-Agg ^{3'}	5'-TTA ^{3'}	5'-Tg g ^{3'}	5'-Tg g ^{3'}	5'-Tg g ^{3'}
3'-primer²	231	2nd I	507	59	58	57
	5'-TgC ^{3'}	5'-AAA ^{3'}	5'-TTg ^{3'}	5'-CTC ^{3'}	5'-ggC ^{3'}	5'-CTC ^{3'}
A*	+	+	+			
B*	+	+	+			
Cw*	+	+	+			
DRB1				+	+	
DRB3				+	+	
DRB5				+		
DQB1					+	
DPB1						+

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.

¹The 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 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.

²The 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. ‘76G’.

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

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

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

INTERPRETATION

Only 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, -DRB4¹ and -DRB5 alleles, i.e. **DRB1*010101 to 1003, DRB3*010101 to DRB3*0303, DRB4*010101 to DRB4*0108, DRB5*010101 to DRB5*0205**, recognized by the HLA Nomenclature Committee in January 2010² will be amplified by the primers in the DR low resolution SSP kit. The HLA-DRB alleles will be grouped into their corresponding serological specificities³.

¹The DRB4*0201N and DRB4*0301N null alleles will not be amplified by the DR low resolution primer set.

²DRB alleles listed on the IMGT/HLA web page 2010-January-15, release 2.28.0, www.ebi.ac.uk/imgt/hla.

³The 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.

The DRB1*1313 and DRB1*1484 alleles yield identical amplification patterns with the DR low resolution primer set. These alleles can be separated by the respective high resolution primer sets.

SPECIFICITY TABLE

DR low resolution primer set

Specificities and sizes of the PCR products of the 24 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^{6,8}	200 bp, 255 bp	515 bp	1	*010101-010205, 0104-0131
2	200 bp	430 bp	1/103	*0103
3	210 bp	430 bp	2, 15	*15010101-1543
4	210 bp	430 bp	16	*160101-160502, 1607-1615
5^{6,11}	120 bp, 220 bp	430 bp	3, 11, 17, 18	*03010101-0352, 1107, 1153, 1525
6^{5,6,11}	80 bp, 210 bp	430 bp	3, 6, 11, 13, 14, 17	*03010101-030108, 0304-0306, 0308-0316, 0318-0320, 0322, 0323, 0325, 0326, 0328, 0330, 0331, 0333, 0334, 0336, 0337, 0343-0348, 0350-0352, 110201-1103, 111101, 111102, 111401, 111402, 1116, 1120, 1121, 1136, 1140, 1141, 1148, 1159, 1163, 116501, 116502, 1168, 1170, 1173, 1176, 1179, 1180, 1183, 1185-1187, 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, 1387-1397, 1416, 1419, 1421, 1482, 1495
7^{5,6}	85 bp, 210 bp	430 bp	3, 6, 11, 13, 14, 1403, 18	*030201-0303, 0327, 0329, 0338, 111301 ^w , 111302 ^w , 1126, 1134, 1315, 1319, 1326, 1344, 1353, 1357, 1385, 1386, 1402-140302, 140601, 140602, 1409, 1412, 1413, 1417-1421, 1424, 1427, 1429, 1430, 143201 ^w , 143202 ^w , 1433, 1440, 1441, 1447-1449, 1451, 1463, 1465 ^w , 1467, 1477, 1478, 1480, 1481, 1483, 1485, 1489, 1494
8^{5,6}	100 bp, 175 bp	430 bp	3, 4	*040101-0489
9⁶	210 bp, 230 bp	430 bp	7, 13, 14	*07010101-070103, 0703-0717, 1317, 1450

Lot No.: **76G**

Lot-specific information

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10⁶	170 bp, 215 bp, 250 bp	515 bp	8, 11, 12, 14	*080101-0819, 0821-0839, 1167, 1204, 1216, 1411, 1415, 1468, 1493
11⁵⁻⁷	85 bp, 135 bp, 180 bp	430 bp	3, 9, 11	*0308, 090102-0909, 1107, 1153
12⁸	205 bp	430 bp	10	*100101-1003
13^{5,6}	100 bp, 170 bp	430 bp	3, 8, 11, 13, 14	*0308, 0831, 110101-1170, 1172- 1189
14^{5,6}	85 bp, 105 bp	430 bp	12	*0832, 120101-1220
15	210 bp	430 bp	6, 11, 13, 14, 1403	*0820, 110101-110405, 110601, 110602, 110801-111202, 111401- 1116, 1118-1121, 1123-1125, 112701-1133, 1135-1151, 115401, 115402, 1156-1166, 1168, 1170, 1172-1181, 1183-1188, 130101- 1308, 1310-1316, 1318-1343, 1345- 1385, 1387-1397, 140301, 140302, 1412, 1416, 1419, 1421, 1422, 1425, 1427, 1440, 1453, 1463, 1467, 1469, 1474, 1477, 1478, 1484, 1485
16⁶	195 bp, 210 bp	430 bp	6, 8, 11, 12, 13, 14	*080101-080203, 080401-0809, 0811, 0816, 0817, 0820-0822, 0824, 0826, 0828, 0831, 0839, 110101- 110602, 1109-111202, 111401-1116, 1120, 1121, 1123-1125, 112701- 1130, 1132, 1133, 1135-1141, 1143, 1144, 1146-1151, 115401-1156, 115801-1163, 116501-1170, 1172, 1174-1178, 1180-1188, 120201- 120204, 1213, 1215, 1216, 1218- 1220, 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, 1391-1393, 1396, 1397, 1415, 1416, 1422, 1424, 1425, 1427, 1437, 1453, 1473
17¹¹	175 bp	430 bp	3, 6, 11, 13, 14, 1403, 17, 18	*03010101-0307, 0309, 031101- 0341, 0343-0345, 0347-0352, 0820, 130101-1316, 1318-1342, 1344, 1346-1366, 1368-1397, 1402- 140302, 140501-140602, 1409, 1412- 1414, 1417-1421, 142301, 142303, 1424, 1427, 1429, 1430, 1433, 1436,

Lot No.: **76G**

Lot-specific information

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				1437, 1440-1445, 1447, 1448, 1451, 1456, 1459, 1463-1465, 1467, 1477, 1478, 1480, 1481, 1483-1485, 1489, 1491, 1494-1496
18^{5,6,10}	100 bp, 140 bp, 155 bp	430 bp	4, 6, 8,13, 14, 1404	*0462, 0469, 0473, 0808, 1169, 1182, 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, 1486-1488, 1490, 1493, DRB4*01030102N
19^{5,6,8,9}	110 bp, 135 bp, 170 bp	430 bp	3, 4, 6, 9, 11, 13, 14, 1404	*0310, 090102-090105, 090201, 090202, 0904-0909, 111301, 111302, 1117, 1152, 1343, 140101-1402, 1404-1411, 1413, 1414, 1416-1418, 1419 ^w , 1420, 1421 ^w , 1422-142303, 1426, 1428-1436, 1438, 1439, 1441, 1443-1452, 1454-1457, 1459-1462, 1464, 1465, 1468, 1470-1476, 1479-1483, 1486-1488, 1490-1496, 1527, 1534
20^{5,6,8}	110 bp, 175 bp, 225 bp	430 bp	2 ^w , 3, 4, 6, 8, 11, 13, 14, 1403, 1404, 16 ^w	*0310, 0809, 0820, 0821, 0832, 0835, 111301, 111302, 1117, 1123, 1125, 1131, 1145, 1152, 1155, 1164, 1189, 1313, 1318, 1343, 1345, 1347, 1355, 140101-140103, 140301-140503, 140701-1408, 1410-1412, 1414-1416, 1418, 1422-142303, 1425-1428, 1431-143202, 1434-1436, 1438-1440, 1442-1445, 1449, 1450, 1453-1465, 1467-1479, 1481, 1482, 1484-1493, 1495, 1496, 1521 ^w , 1604 ^w
21^{5,6,10,11}	100 bp, 165 bp, 240 bp	430 bp	52	DRB3*01010201-0114, DRB3*0201-0225, DRB3*030101-0303
22	215 bp	430 bp	53	DRB4*01010101-0108
23	175 bp	430 bp	51	DRB5*010101-0114, DRB5*0202-0205
24¹²	-	-		Negative control

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 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, 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 inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DR low resolution typing.

In addition, well number 10 contains 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.

³The 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, the information available at the www.worldmarrow.org web site, the expert-assigned serological grouping in Tissue Antigens (2009) 73:95-170, and have also inferred the serological grouping from the naming of the sequence-defined allele.

⁴For 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.

The DRB1*1313 and DRB1*1484 alleles yield identical amplification patterns with the DR low resolution primer set. These alleles can be separated by the respective high resolution primer sets.

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

⁶Individual alleles can give rise to two differently sized specific PCR fragments in primer mix 1, 5 to 11, 13, 14, 16 and 18 to 21.

⁷Primer mix 11 may give rise to nonspecific amplifications.

⁸Primer mixes 1, 12, 19 and 20 may give rise a primer oligomer formation.

⁹Primer 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.

¹⁰The DRB4*01030102N allele is amplified by the primer pairs in well No. 18 and 22, whereas the DRB4*0201N and DRB4*0301N null alleles are not amplified by these primer pairs.

¹¹Due to sharing of sequence motifs in codon 38, DRB3*0114 will also be amplified in primer mixes 5, 6 and 17 in addition to primer mix 21.

¹²Primer mix 24 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by control primer pairs. PCR product sizes range from 75 to 200 base pairs. The PCR product generated by the control primer pair is 430 base pairs.

‘w’, might be weakly amplified.

‘?’, nucleotide sequence information not available for the primer matching sequence.

INTERPRETATION TABLE

DR low resolution SSP typing

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

		Well ⁶											
		1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product(s)		200 255	200	210	210	120 220	80 210	85 210	100 175	210 230	170 215	85 135	205
Length of int. pos. control ¹											250	180	
5'-primer(s) ²		515 (129)	430 (129)	430 (126)	430 (126)	430 (125)	430 (125)	430 (125)	430 (125)	430 (127)	515 (133)	430 (165)	430 (178)
		14 (129) 5' -gAA 3'	14 (129) 5' -gAA 3'	13 (126) 5' -Agg 3'	13 (126) 5' -Agg 3'	13 (125) 5' -gTC 3'	13 (125) 5' -gTC 3'	13 (125) 5' -gTC 3'	13 (125) 5' -ACA 3'	14 (127) 5' -ATA 3'	16 (133) 5' -gTT 3'	26 (165) 5' -TAT 3'	31 (178) 5' -gCg 3'
				13 (126) 5' -Aag 3'	13 (126) 5' -Aag 3'	47 (227) 5' -gTT 3'	16 (133) 5' -gTT 3'		13 (125) 5' -ACC 3'	14 (127) 5' -ATA 3'	16 (133) 5' -gTT 3'	58 (261) 5' -gAg 3'	
									13 (125) 5' -ATA 3'	16 (133) 5' -gTT 3'			
									13 (125) 5' -gTC 3'				
3'-primer(s) ³		67 (286) 5' -gAg 3'	67 (286) 5' -gAT 3'	67 (286) 5' -gAT 3'	67 (286) 5' -gAA 3'	73 (305) 5' -ggC 3'	26 (164) 5' -ggT 3'	28 (171) 5' -CTC 3'	33 (184) 5' -gTg 3'	71 (298) 5' -CTC 3'	58 (260) 5' -CCT 3'	57 (257) 5' -CgA 3'	86 (344) 5' -CAC 3'
		67 (286) 5' -gAg 3'		70 (295) 5' -CTg 3'	67 (286) 5' -gAg 3'	73 (305) 5' -ggC 3'	71 (299) 5' -gCT 3'	70 (295) 5' -CTg 3'	58 (260) 5' -Cgg 3'	73 (305) 5' -ggC 3'	74 (307) 5' -Cag 3'	73 (305) 5' -ggC 3'	86 (344) 5' -CCA 3'
		67 (286) 5' -gAT 3'		71 (298) 5' -CgC 3'	72 (303) 5' -gCg 3'	74 (308) 5' -CCC 3'				77 (317) 5' -AAT 3'	86 (344) 5' -CAC 3'	78 (319) 5' -CAC 3'	
		71 (299) 5' -gCg 3'		71 (299) 5' -gCT 3'						78 (319) 5' -CAC 3'			
		86 (344) 5' -CCA 3'		73 (305) 5' -ggC 3'									
Well No.	DR	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 ⁶														
13	14	15	16	17	18	19	20	21	22	23	24			
100	85	210	195	175	100	110	110	100	215	175			Length of spec. PCR product(s)	
170	105		210		140	135	175	165						
					155	170	225	240						
430	430	430	430	430	430	430	430	430	430	430			Length of int. pos. control ¹	
13	16	10	10	13	5	26	13	10	28	13				
(125)	(133)	(116)	(116)	(125)	(101)	(164)	(125)	(116)	(170)	(125)			5'-primer(s) ²	
5'-gTC ^{3'}	5'-gTT ^{3'}	5'-gCT ^{3'}	5'-gCT ^{3'}	5'-gTC ^{3'}	5'-CAA ^{3'}	5'-gTA ^{3'}	5'-gTC ^{3'}	5'-gCT ^{3'}	5'-gAT ^{3'}	5'-gTA ^{3'}				
16		13	13		37	34	34	10					Negative Control	
(133)		(125)	(125)		(197)	(189)	(189)	(116)						
5'-gTC ^{3'}		5'-gTC ^{3'}	5'-gTC ^{3'}		5'-gTT ^{3'}	5'-CAG ^{3'}	5'-CAG ^{3'}	5'-gCT ^{3'}						
38			16		37			57						
(200)			(133)		(197)			(258)						
5'-CgT ^{3'}			5'-gTT ^{3'}		5'-gTA ^{3'}			5'-gTC ^{3'}						
			16											
			(133)											
			5'-gTC ^{3'}											
58	30	70	67	58	42	57	57	51	87	57				
(260)	(175)	(295)	(286)	(260)	(213)	(257)	(257)	(239)	(346)	(258)			3'-primer(s) ³	
5'-CCT ^{3'}	5'-gTg ^{3'}	5'-gTC ^{3'}	5'-gAA ^{3'}	5'-Cgg ^{3'}	5'-TCA ^{3'}	5'-CAG ^{3'}	5'-CAG ^{3'}	5'-CCC ^{3'}	5'-CTC ^{3'}	5'-gCg ^{3'}				
58	38	71	71	58	57	70	60	77	87	58				
(260)	(199)	(299)	(298)	(260)	(257)	(295)	(265)	(317)	(346)	(260)				
5'-CCT ^{3'}	5'-CAG ^{3'}	5'-gCT ^{3'}	5'-CgC ^{3'}	5'-CAG ^{3'}	5'-CAG ^{3'}	5'-CTg ^{3'}	5'-gTg ^{3'}	5'-AAT ^{3'}	5'-CTT ^{3'}	5'-CCT ^{3'}				
58			71		71	70	70							
(260)			(298)		(298)	(296)	(296)							
5'-CCT ^{3'}			5'-CTC ^{3'}		5'-CgC ^{3'}	5'-TCC ^{3'}	5'-TCC ^{3'}							
							74							
							(307)							
							5'-CAG ^{3'}							
13	14	15	16	17	18	19	20	21	22	23	24	DR	Well No.	

Lot No.: **76G**

Lot-specific information

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Well No.	DR	1	2	3	4	5	6	7	8	9	10	11	12
DRB1 allele ⁴	ser ⁵												
*010101-010205, 0104-0131	1	1											
*0103	1/103		2										
*03010101-030108, 0304-0306, 0309, 031101-0316, 0318-0320, 0322, 0323, 0325, 0326, 0328, 0330, 0331, 0333, 0334, 0336, 0337, 0343-0345, 0347, 0348, 0350-0352	3, 17					5	6						
*030201-0303, 0327, 0329, 0338	3, 18					5		7					
*0307, 0317, 0321, 0324, 0332, 0335, 0339-0341, 0349	3					5							
*0308	3					5	6					11	
*0310	3					5	6						
*0342	-					5							
*0346	-					5	6						
*040101-0461, 0463-0468, 0470-047202, 0474-0489	3, 4								8				
*0462, 0469, 0473	4								8				
*07010101-070103, 0703-0717	7, null									9			
*080101-080203, 080401-0807, 0811, 0816, 0817, 0822, 0824, 0826, 0828, 0839	8										10		
*080302, 0810, 0812-0815, 0818, 0819, 0823, 0825, 0827, 0829-083002, 0833, 0834, 0836-0838	8										10		
*0808	8										10		
*0809, 0821, 1415	8										10		
*0820, 1318, 1347, 1355	-/13												
*0831, 1167	8, 11										10		
*0832	-										10		
*0835	-										10		
*090102-090105, 090201, 090202, 0904-0909	9											11	
*090106, 0903	9											11	
*100101-1003	10												12
Well No.	ser ⁵	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **76G**

Lot-specific information

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13	14	15	16	17	18	19	20	21	22	23	24	DR ser ⁵	Well No. DRB1 allele ⁴
												1	*010101-010205, 0104-0131
												1/103	*0103
				17								3, 17	*03010101-030108, 0304-0306, 0309, 031101-0316, 0318-0320, 0322, 0323, 0325, 0326, 0328, 0330, 0331, 0333, 0334, 0336, 0337, 0343-0345, 0347, 0348, 0350-0352
				17								3, 18	*030201-0303, 0327, 0329, 0338
				17								3	*0307, 0317, 0321, 0324, 0332, 0335, 0339-0341, 0349
13												3	*0308
						19	20					3	*0310
												-	*0342
												-	*0346
												3, 4	*040101-0461, 0463-0468, 0470-047202, 0474-0489
					18							4	*0462, 0469, 0473
												7, null	*07010101-070103, 0703-0717
			16									8	*080101-080203, 080401-0807, 0811, 0816, 0817, 0822, 0824, 0826, 0828, 0839
												8	*080302, 0810, 0812-0815, 0818, 0819, 0823, 0825, 0827, 0829-083002, 0833, 0834, 0836-0838
			16		18							8	*0808
			16				20					8	*0809, 0821, 1415
		15	16	17			20					-/13	*0820, 1318, 1347, 1355
13			16									8, 11	*0831, 1167
	14						20					-	*0832
							20					-	*0835
						19						9	*090102-090105, 090201, 090202, 0904-0909
												9	*090106, 0903
												10	*100101-1003
13	14	15	16	17	18	19	20	21	22	23	24	DR ser ⁵	Well No.

Negative Control

Lot No.: **76G**

Lot-specific information

www.olerup-ssp.com

Well No.	ser ⁵	1	2	3	4	5	6	7	8	9	10	11	12
*110101-110111, 110401-110405, 110601, 110602, 1109-111002, 111201, 111202, 1115, 1124, 112701-1130, 1132, 1133, 1135, 1137-1139, 1143, 1144, 1146, 1147, 1149-1151, 115401, 115402, 1156, 115801, 115802, 1160-1162, 1166, 1172, 1174, 1175, 1177, 1178, 1181, 1184, 1188	11												
*110201-1103, 111101, 111102, 111401, 111402, 1116, 1120, 1121, 1136, 1140, 1141, 1148, 1159, 1163, 116501, 116502, 1168, 1170, 1176, 1180, 1183, 1185-1187	11, 13						6						
*1105	11												
*1107, 1153	11					5						11	
*110801, 110802, 1118-111902, 1142, 1157	11												
*111301, 111302	11							w					
*1117, 1152	11, 14												
*1122	-												
*1123, 1125	11												
*1126, 1134	11							7					
*1131, 1145, 1164	11												
*1155	11												
*1169, 1182	-												
*1173, 1179	-						6						
*1189	-												
*120101-120103, 120302, 1205-1212, 1214, 1217	12												
*120201-120204, 1213, 1215, 1218-1220	12												
*1204	12										10		
*1216	-										10		
Well No.	ser ⁵	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **76G**

Lot-specific information

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13	14	15	16	17	18	19	20	21	22	23	24	ser ⁵	Well No.
13		15	16									11	*110101-110111, 110401-110405, 110601, 110602, 1109-111002, 111201, 111202, 1115, 1124, 112701-1130, 1132, 1133, 1135, 1137-1139, 1143, 1144, 1146, 1147, 1149-1151, 115401, 115402, 1156, 115801, 115802, 1160-1162, 1166, 1172, 1174, 1175, 1177, 1178, 1181, 1184, 1188
13		15	16									11, 13	*110201-1103, 111101, 111102, 111401, 111402, 1116, 1120, 1121, 1136, 1140, 1141, 1148, 1159, 1163, 116501, 116502, 1168, 1170, 1176, 1180, 1183, 1185-1187
13			16									11	*1105
13												11	*1107, 1153
13		15										11	*110801, 110802, 1118-111902, 1142, 1157
13						19	20					11	*111301, 111302
13						19	20					11, 14	*1117, 1152
13												-	*1122
13		15	16				20					11	*1123, 1125
13												11	*1126, 1134
13		15					20					11	*1131, 1145, 1164
13			16				20					11	*1155
13			16		18							-	*1169, 1182
13		15										-	*1173, 1179
13							20					-	*1189
	14											12	*120101-120103, 120302, 1205-1212, 1214, 1217
	14		16									12	*120201-120204, 1213, 1215, 1218-1220
	14											12	*1204
	14		16									-	*1216
13	14	15	16	17	18	19	20	21	22	23	24	ser ⁵	Well No.

Lot No.: **76G**

Lot-specific information

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Well No.	ser ⁵	1	2	3	4	5	6	7	8	9	10	11	12
*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, 1391-1393, 1396, 1397	11, 13, 14						6						
*130202, 130301-130304, 1310, 133301-133303, 1337, 1366, 1381, 1388-1390, 1394, 1395	13						6						
*130501, 130502, 130701, 130702, 131101, 131102, 131401-131403, 132101, 132102, 1342, 1346, 1349-135002, 1362	6, 11, 13												
*1306, 1312, 1325, 1330, 1356, 1358, 1360, 1377, 1382	6, 11, 13												
*1309	13												
*1313, 1484	13, -												
*1315, 1319, 1353, 1357	13						6	7					
*1317	13						6			9			
*1326	14							7					
*1343	13						6						
*1344, 1386	-							7					
*1345	13						6						
*1367	13												
*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, 1486-1488, 1490	4, 6, 14, 1404												
*140103, 1408, 142302, 1434, 1472, 1492N	14												
Well No.	ser ⁵	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **76G**

Lot-specific information

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13	14	15	16	17	18	19	20	21	22	23	24	ser ⁵	Well No.
		15	16	17								11, 13, 14	*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, 1391-1393, 1396, 1397
		15		17								13	*130202, 130301-130304, 1310, 133301-133303, 1337, 1366, 1381, 1388- 1390, 1394, 1395
		15	16	17								6, 11, 13	*130501, 130502, 130701, 130702, 131101, 131102, 131401-131403, 132101, 132102, 1342, 1346, 1349- 135002, 1362
		15		17								6, 11, 13	*1306, 1312, 1325, 1330, 1356, 1358, 1360, 1377, 1382
			16	17								13	*1309
		15		17			20					13, -	*1313, 1484
		15	16	17								13	*1315, 1319, 1353, 1357
			16									13	*1317
		15	16	17								14	*1326
		15	16			19	20					13	*1343
				17								-	*1344, 1386
		15	16		18		20					13	*1345
		15	16									13	*1367
		15		17								-	*1385
					18	19	20					4, 6, 14, 1404	*140101, 140102, 1404, 140701, 140702, 1410, 1426, 1428, 1431, 1435, 1438, 1439, 1454, 1455, 1457, 1460-1462, 1470, 1471, 1475, 1476, 1479, 1486-1488, 1490
						19	20					14	*140103, 1408, 142302, 1434, 1472, 1492N
13	14	15	16	17	18	19	20	21	22	23	24	ser ⁵	Well No.

Lot No.: **76G**

Lot-specific information

www.olerup-ssp.com

Well No.	ser ⁵	1	2	3	4	5	6	7	8	9	10	11	12
*1402, 140601, 140602, 1409, 1413, 1417, 1420, 1429, 1430, 1433, 1441, 1447, 1448, 1451, 1480, 1483, 1494	6, 14							7					
*140301, 140302, 1412, 1440, 1463, 1467, 1477, 1478, 1485	6, 14, 1403							7					
*140501-140503, 1414, 142301, 142303, 1436, 1443-1445, 1456, 1459, 1464, 1491, 1496	14												
*1411	14									10			
*1416	6						6						
*1418, 1481	14							7					
*1419, 1421	14						6	7					
*1422	14												
*1424	14							7					
*1425, 1453	6, 13, 14												
*1427	14							7					
*143201, 143202	14							w					
*1437	14												
*1442	-												
*1446, 1452	14												
*1449	14							7					
*1450	14									9			
*1458	14												
*1465	6							w					
*1468, 1493	14									10			
*1469	-												
*1473	-												
*1474	-												
*1482	-						6						
*1489	-							7					
*1495	-						6						
*15010101-1520, 1522- 1524, 1526, 1528-1533, 1535-1543	2, 15, Null			3									
*1521	2			3									
*1525	-			3		5							
*1527, 1534	-			3									
*160101-1603, 160501, 160502, 1607-1615	16				4								
*1604	16				4								
Well No.	ser ⁵	1	2	3	4	5	6	7	8	9	10	11	12

Lot No.: **76G**

Lot-specific information

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13	14	15	16	17	18	19	20	21	22	23	24	ser ⁵	Well No.
				17		19						6, 14	*1402, 140601, 140602, 1409, 1413, 1417, 1420, 1429, 1430, 1433, 1441, 1447, 1448, 1451, 1480, 1483, 1494
		15		17			20					6, 14, 1403	*140301, 140302, 1412, 1440, 1463, 1467, 1477, 1478, 1485
				17		19	20					14	*140501-140503, 1414, 142301, 142303, 1436, 1443-1445, 1456, 1459, 1464, 1491, 1496
						19	20					14	*1411
		15	16		18	19	20					6	*1416
				17		19	20					14	*1418, 1481
		15		17		w						14	*1419, 1421
		15	16		18	19	20					14	*1422
			16	17								14	*1424
		15	16		18		20					6, 13, 14	*1425, 1453
		15	16	17			20					14	*1427
					18	19	20					14	*143201, 143202
			16	17	18							14	*1437
				17			20					-	*1442
						19						14	*1446, 1452
					18	19	20					14	*1449
					18	19	20					14	*1450
					18		20					14	*1458
				17		19	20					6	*1465
					18	19	20					14	*1468, 1493
		15			18		20					-	*1469
			16		18	19	20					-	*1473
		15			18	19	20					-	*1474
					18	19	20					-	*1482
				17			20					-	*1489
				17		19	20					-	*1495
												2, 15, Null	*15010101-1520, 1522-1524, 1526, 1528-1533, 1535-1543
							w					2	*1521
												-	*1525
						19						-	*1527, 1534
												16	*160101-1603, 160501, 160502, 1607-1615
							w					16	*1604
13	14	15	16	17	18	19	20	21	22	23	24	ser ⁵	Well No.

Negative Control

Well No.	ser ⁵	1	2	3	4	5	6	7	8	9	10	11	12
DRB3*01010201-0114, DRB3*0201-0225, DRB3*030101-0303	52												
DRB4*01010101- 01030101, 010302-0108	51												
DRB4*01030102N	Null												
DRB5*010101-0114, DRB5*0202-0205	51												
Well No.	ser ⁵	1	2	3	4	5	6	7	8	9	10	11	12

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs, for most wells, or a band of 515 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DR low resolution typing.

In addition, well number 10 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

²The codon, and in parenthesis the nucleotide, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given. Codon and nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

³The codon, and in parenthesis the nucleotide, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Codon and nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

Lot No.: **76G**

Lot-specific information

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13	14	15	16	17	18	19	20	21	22	23	24	ser ⁵	Well No.
								21				52	DRB3*01010201-0114, DRB3*0201-0225, DRB3*030101-0303
									22			51	DRB4*01010101- 01030101, 010302-0108
					18				22			Null	DRB4*01030102N
										23		51	DRB5*010101-0114, DRB5*0202-0205
13	14	15	16	17	18	19	20	21	22	23	24	ser ⁵	Well No.

⁴The 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*1171 allele has been shown to be identical to DRB1*110201.
The sequence of the DRB1*120301 allele has been shown to be identical to DRB1*1201.
The DRB1*1466 allele has been renamed DRB1*143202.
The sequence of the DRB1*1606 allele has been shown to be identical to DRB1*1605.
The sequence of the DRB3*010101 allele has been shown to be identical to DRB3*01010201.
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.
The DRB1*1313 and DRB1*1484 alleles yield identical amplification patterns in the DR low kit. These alleles can be separated by the respective high resolution primer sets.
Due to sharing of sequence motifs in codon 38, DRB3*0114 will also be amplified in primer mixes 5, 6 and 17 in addition to primer mix 21.

⁵The 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, the expert-assigned serological grouping in Tissue Antigens (2009) 73:95-170 and have also inferred the serological grouping from the naming of the sequence-defined allele.

⁶Primer mix 24 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by control primer pairs. PCR product sizes range from 75 to 200 base pairs. The PCR product generated by the control primer pair is 430 base pairs.

'ser', serological HLA specificity.

'w', may be weakly amplified.

[illegible]

CELL LINE VALIDATION SHEET												
DR low resolution primer set												
					Prod. No.:	Well						
						17	18	19	20	21	22	23
						200960317	200968518	200963419	200960320	200968521	201070822	200960323
	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	9025	DEU	*0401			-	-	-	-	-	+	-
9	9026	YAR	*0402			-	-	-	-	-	+	-
10	9107	LKT3	*0405			-	-	-	-	-	+	-
11	9051	PITOUT	*0701			-	-	-	-	-	+	-
12	9052	DBB	*0701			-	+	-	-	-	+	-
13	9004	JESTHOM	*0101			-	-	-	-	-	-	-
14	9071	OLGA	*0802			-	-	-	-	-	-	-
15	9075	DKB	*0901			-	-	+	-	-	+	-
16	9037	SWEIG007	*1101			-	-	-	-	+	-	-
17	9292	CTM39539540	*0301	*1301		+	-	-	-	+	-	-
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:** 76G**Expiry date:** 2012-February-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	2009-685-01	9	2009-603-09	17	2009-603-17
2	2009-603-02	10	2009-603-10	18	2009-685-18
3	2009-685-03	11	2009-603-11	19	2009-634-19
4	2009-603-04	12	2009-603-12	20	2009-603-20
5	2009-634-05	13	2009-603-13	21	2009-685-21
6	2009-603-06	14	2009-603-14	22	2010-708-22
7	2009-603-07	15	2009-603-15	23	2009-603-23
8	2009-634-08	16	2009-603-16		

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

The reactivities of additional 3'-primers in primer solutions 1, 3, 9 to 12, 18 and 20 were tested by separately adding another 5'-primer. Additional 5'-primers in primer solutions 6, 9, 11, 15, 16, 18 and 22 were tested by separately adding another 3'-primer. One or more of the 5'-primers in primer solutions 3, 4, 8 to 10, 13 and 16 and one or two of the 3'-primers in primer solutions 1, 3, 13 and 22 were not possible to test.

The negative control primer pairs, **Production No. 2009-614-01**, can detect contamination with PCR products diluted 10^{-7} .

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

Date of approval: 2010-July-02

Approved by:

Quality Control Supervisor

Lot No.: **76G**

Lot-specific information

www.olerup-ssp.com

Declaration of Conformity

Product name: *Olerup SSP*[®] DR low resolution
Product number: 101.101-48/12
Lot number: 76G

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 9001:2008 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.

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Saltsjöbaden, Sweden
2010-July-02

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