

Olerup SSP™ DQ-DR SSP Combi Tray

Product number: 101.704-48/12 – licensed for PCR
101.704-48u/12u – not licensed for PCR
Lot number: X80
Expiry date: 2009-May-01
Number of tests: 48 tests – Product No. 101.704-48
12 tests – Product No. 101.704-12
Number of tubes per test: 31 + 1
Storage - pre-aliquoted primers: dark at -20°C
- PCR Master Mix: -20°C

This Product Description is only valid for Lot No. X80.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP™ DQ-DR SSP COMBI TRAY LOT

The DQ low resolution specificity and interpretation tables have been updated for the HLA-DQB1 alleles described since the previous *Olerup SSP™* DQ-DR Combi Tray lot was made (**Lot No. V95**).

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

Tube	5'-primer	3'-primer	rationale
4	Modified	Modified	Increased yield of specific PCR product
5	Modified	Modified	Increased yield of specific PCR product
6	Modified	Modified	Increased yield of specific PCR product
7	Modified	Modified	Increased yield of specific PCR product
8	Modified	Modified	Increased yield of specific PCR product

Lot No.: **X80**

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The DR low resolution specificity and interpretation tables have been updated for the HLA-DRB1 alleles described since the previous *Olerup* SSP™ DQ-DR Combi Tray lot was made (**Lot No. V95**).

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

Tube	5'-primer	3'-primer	rationale
9	Modified	Modified	Increased yield of specific PCR product.
13	-	Modified	Primer added for the DRB1*030105 allele.
16	Removed, added	Added	Primers added for the DRB1*0463 and *0464 alleles.
23	-	Modified	Increased specificity of specific primer.
24	Added	-	Primer added for the DRB1*1105 allele.
26	Modified	-	Increased yield of specific PCR product.

Well **32** 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.

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						+

¹The 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. Nucleotide numbering as in *Tissue Antigens* 1998, 51:II, 417-466. 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, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as in *Tissue Antigens* 1998, 51:II, 417-466. The sequence of the 3 terminal nucleotides of the primer is given.

PRODUCT DESCRIPTION

DQ-DR SSP Combi Tray

CONTENT

The primer set contains 5'- and 3'-primers for grouping the DQB1 alleles in to the serological groups DQ2 to DQ9.

The primer set contains 5'- and 3'-primers for grouping the DRB1*0101 to DRB1*1001 alleles into the corresponding serological groups DR1 to DR18 as well as primer pairs for recognizing the DRB3, DRB4 and DRB5 groups of alleles. The primer set also contains negative control primer pairs, that will amplify more than 90% of the Olerup SSP™ HLA Class I, DRB, DQB1 and DPB1 amplicons as well as the amplicons generated by control primer pairs.

The primer solutions are pre-aliquoted into 0.2 ml PCR tubes. Each tube in the set contains a dried primer solution consisting of a specific primer mix, i.e. allele- and group-specific primers as well as a **control primer pair** matching non-allelic sequences.

PCR Master Mix complete with Taq, Taq polymerase, nucleotides, buffer, glycerol and cresol red, as well as PCR lids are included in the licensed kit.

PCR Master Mix without Taq, nucleotides, buffer, glycerol and cresol red, as well as PCR lids are included in the unlicensed kit.

31 + 1 PCR reactions with a reaction volume of 10 µl are performed per sample.

Note: The pellets in the tubes may vary in form and colour. This does not affect the performance of the product.

Please note that DQB1 amplifications usually are somewhat less pronounced than e.g. DRB and DQA1 amplifications even when using the same DNA preparation and exactly the same experimental procedures.

PLATE LAYOUT

Each test consists of 32 PCR reactions in a 32 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
25	26	27	28	29	30	31	32

Wells 1 to 8 – DQ low resolution primers.

Wells 9 to 31 – DR low resolution primers.

Well 32 – Negative Control.

The 32 well cut PCR plate is marked with 'DQ-DR R60'.

Well No. 1 is marked with '1'.

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

Please note: When removing each 32 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Only the DQB1 alleles will be amplified by the 8 tubes of the DQ low resolution primer set, **tubes 1 to 8**. Thus, the interpretation of DQ low resolution typings is not influenced the DQB2 and DQB3 genes.

Only HLA-DRB alleles will be amplified by the 23 tubes of the DR low resolution primer set, **tubes 9 to 31**. Thus, the interpretation of DR low resolution typings is not influenced by other HLA class II genes.

UNIQUELY IDENTIFIED ALLELES

All the DQB1 alleles, i.e. **DQB1*050101 to 0505, DQB1*060101 to 0630, DQB1*020102 to 0205, DQB1*030101 to 0320 and DQB1*0401 to 0402**, recognized by the HLA Nomenclature Committee in April 2007¹ will be amplified by the primers in the DQ low resolution SSP primer set, **tubes 1 to 8**. The DQB1 alleles will be grouped into their corresponding serological specificities, i.e.:

DQ5(1) = DQB1*050101-0505²
DQ6(1) = DQB1*060101-0630²
DQ2 = DQB1*020101-0205
DQ3 = DQB1*030101-0320²
DQ7(3) = DQB1*030101-030103, DQB1*0304
DQ8(3) = DQB1*030201, DQB1*030501, DQB1*0310
DQ9(3) = DQB1*030302
DQ4 = DQB1*0401, DQB1*0402

¹**Nomenclature for factors of the HLA system, 1998.** *Tissue Antigens* 1999; **53**: 407-446.

HLA-DQB1 alleles listed on the IMGT/HLA web page 2007-April-12, release 2.17.0, www.ebi.ac.uk/imgt/hla.

²The serological split of the DQB1*0505, DQB1*0606 to 0608 alleles, the DQB1*0610 to 0630, the DQB1*030202-030204, DQB1*030303 and the DQB1*030502 to 0320 alleles is not known. In this table we have inferred the serological grouping from the naming of the sequence-defined allele.

All the HLA-DRB1¹, -DRB3, -DRB4² and -DRB5 alleles, i.e. **DRB1*010101 to 100102, DRB3*010101 to DRB3*0303, DRB4*01010101 to DRB4*0107, DRB5*010101 to DRB5*0205**, recognized by the HLA Nomenclature Committee in April 2007³ will be amplified by the primers in the DR low resolution SSP kit. The HLA-DRB alleles will be grouped into their corresponding serological specificities⁴.

¹The nucleotide sequences of the DRB1*0334, *110404, *1161, *1163 and *1377 are not yet retrievable.

²The DRB4*0201N and DRB4*0301N null alleles will not be amplified by the DR low resolution primer set.

³**Nomenclature for factors of the HLA system, 1998.** *Tissue Antigens* 1999; **53**: 407-446 and DRB1, DRB3, DRB4 and DRB5 alleles listed on the IMGT/HLA web page 2007-April-12, release 2.1670, www.ebi.ac.uk/imgt/hla.

⁴The DRB1*0809, DRB1*0821 and DRB4*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.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The ambiguous HLA-DQ homo- and heterozygous combinations of allele groups based on the two first digits of the allele designation are available in the Score™ interpretation software. (These ambiguities can be resolved by the Olerup SSP™ DQB1 high resolution SSP sets.)

The ambiguous HLA-DR homo- and heterozygous combinations of allele groups based on the two first digits of the allele designation are available in the Score™ interpretation software. (In most cases these ambiguities can be resolved by the Olerup SSP™ DRB1 high resolution SSP sets.)

LICENSES

101.704-48/12 – licensed for PCR.

Notice to purchaser: Limited License.

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101.704-48/12 and 101.704-48u/12u

These products use ARMSTM technology and is sold under license from Zeneca Limited. ARMS is the subject of European Patent No. 0332435, US Patent No. 5595890 and corresponding world-wide patents. ARMS is a trademark of Zeneca Limited.

GUARANTEE

Olerup SSP AB guarantees that the primers in the DQ-DR SSP Combi Tray have the specificities given in the Specificity and Interpretation Tables of the product insert and in the GenoVision version of the HELMBERG-SCORETM software. When stored at –20°C, the dried primers are stable for 22 months from the date of manufacture.

When stored at –20°C, the PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* are stable for 24 months from the date of manufacture. The kit is shipped at ambient temperature.

PROTOCOL

DNA EXTRACTION

Extracted, highly pure DNA is needed for SSP typings. We recommend isolation of DNA using GenoPrep B200 or GenoPrep B350 cartridges on the GenoM™-6 robotic workstation (GenoVision Europe Tel: +43 1 710 15 00 or GenoVision Inc. USA Tel: +1 610 430 88 41; <http://www.genovision.com>). Using GenoM™-6-extracted DNA ACD, EDTA and heparinised blood can be used as starting material. Because of its high purity, GenoM™-6-extracted DNA can be diluted when used in combination with *Olerup* SSP™ products. The recommended DNA concentration is 15 ng/μl.

Alternatively – BUT DO NOT USE HEPARINISED BLOOD WITH THESE METHODS - the DNA can be extracted using trimethylammoniumbromide salts (DTAB/CTAB) or by salting out. Dissolve the extracted DNA in dH₂O.

IMPORTANT:

Optimal DNA concentration using: GenoM™-6-extracted DNA, 15 ng/μl.

DNA extracted by other methods, 30 ng/μl.

Concentration exceeding 50 ng/μl will increase the risk for nonspecific amplifications and weak extra bands, especially for HLA Class I high resolution SSP typings.

PCR AMPLIFICATION

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For one DQ-DR typing, begin by adding to well No. 32, i.e. the well with the negative control primer pairs:

7 μl dH₂O

3 μl PCR Master Mix complete with *Taq*

then add at room temperature in a 0.5 ml tube:

37 x 2 μl = 74 μl DNA (30 ng/μl)

37 x 3 μl = 111 μl PCR Master Mix complete with *Taq* – mix well
before taking your aliquot

37 x 5 μl = 185 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-H₂O mixture into each of well 1 to 31 of a DQ-DR typing. **Well No. 1 of the 32 well PCR plate is marked with '1'**. Close the 32 well PCR plate with the provided lids.

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For one DQ-DR typing, begin by adding at room temperature in a 0.5 ml tube:

38 x 3 μl = 114 μl PCR Master Mix without *Taq* – mix well before
taking your aliquot

3 μl *Taq* polymerase (5 units/μl)

Mix well, dispense 3 μl of the PCR Master Mix-*Taq* mixture from the 0.5 ml tube into well No. 32, i.e. the well with the negative control primer pairs. Then add 7 μl dH₂O to well 32.

Lot No.: **X80**

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Then add at room temperature to the 0.5 ml tube with the PCR Master Mix-*Taq* mixture:

$$37 \times 2 \mu\text{l} = 74 \mu\text{l DNA (30 ng}/\mu\text{l)}$$

$$37 \times 5 \mu\text{l} - 3 \mu\text{l} = 182 \mu\text{l dH}_2\text{O}$$

Mix well, dispense 10 μl of the DNA-PCR Master Mix-*Taq*-H₂O mixture into wells 1 to 31 of a DR –DQ typing. **Well No. 1 of the 32 well PCR plate is marked with '1'**. Close the 32 well PCR plate with the provided lids.

Use a 96 well thermal cycler with a heated lid. The temperature gradient across the heating block should be < 1°C.

PCR cycling parameters:

1. 1 cycle	94°C	2 min	denaturation
2. 10 cycles	94°C	10 sec.	denaturation
	65°C	60 sec.	annealing and extension
3. 20 cycles	94°C	10 sec.	denaturation
	61°C	50 sec.	annealing
	72°C	30 sec.	extension

The same PCR cycling parameters are used for all the Olerup SSP kits.

AGAROSE GEL ELECTROPHORESIS

Prepare a 2% (w/v) agarose gel in 0.5 x TBE buffer. Dissolve the agarose by boiling in a microwave oven. Let the gel solution cool to 60°C. Stain the gel prior to casting with ethidium bromide (10 mg/ml), 5 μl per 100 ml gel solution. For maximal ease of handling use our ethidium bromide dropper bottles (Product No. 103.301-10), 1 drop of ethidium bromide solution per 50-75 ml of gel. **Note: Ethidium bromide is a powerful carcinogen.**

Load the PCR products, preferably using an 8-channel pipette. Load a DNA size marker (100 base pair ladder, Product No. 103.201-100) in one well per row.

Run the gel in 0.5 x TBE buffer, without re-circulation of the buffer, for 15-20 minutes at 8-10 V/cm.

DOCUMENTATION AND INTERPRETATION

Put the gel on a UV transilluminator and document by photography.

Record the presence and absence of specific PCR products. The relative lengths of the specific PCR products are helpful in the interpretation of the results.

Record the presence and relative lengths of the internal positive control bands. The differently sized control bands will help in the correct orientation of the typing as well as in kit identification.

Lanes without either control band or specific PCR products should be repeated.

Interpret the typings with the **lot-specific Interpretation and Specificity Tables**.

INTERPRETATION SOFTWARE

The interpretation software (Product No. 110.101) can be helpful in the interpretation of the typings.

PCR MASTER MIXES

The PCR Master Mix complete with *Taq* contains:

<i>Taq</i> polymerase	0.4 unit per 10 µl SSP reaction
nucleotides	final concentration of each dNTP is 200 µM
PCR buffer	final concentrations: 50 mM KCl, 1.5 mM MgCl ₂ , 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin
glycerol	final concentration of glycerol is 5%
cresol red	final concentration of cresol red is 100 µg/ml

The same PCR Master Mix complete with *Taq* is used for all the licensed *Olerup* SSP kits.

The PCR Master Mix without *Taq* contains:

nucleotides	final concentration of each dNTP is 200 µM
PCR buffer	final concentrations: 50 mM KCl, 1.5 mM MgCl ₂ , 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin
glycerol	final concentration of glycerol is 5%
cresol red	final concentration of cresol red is 100 µg/ml

The same PCR Master Mix without *Taq* is used for all the unlicensed *Olerup* SSP kits.

The PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* can be shipped at ambient temperature.

When stored at –20°C, the PCR Master Mix complete with *Taq* and the PCR Master Mix without *Taq* are stable for 24 months from the date of manufacture.

Vials with the PCR Master Mixes can be kept at +4°C for 4 weeks, but the PCR Master Mixes are then no longer stable for 24 months.

SPECIFICITY TABLE

DQ low resolution primer set

Specificities and sizes of the PCR products of the 8 primer mixes of the DQ low resolution primer set

Primer Mix	Approx. size of spec. PCR product ¹	Size of control band ²	DQ serology ³	Amplified DQB1 alleles ⁴
1	220 bp	515 bp	5	050101-0505
2	220, 270 bp	430 bp	6	060101-0630
3	205 bp	430 bp	2	020101-0205
4	130, 145 bp	515 bp	2, 3, 6, 8	020101-0202, 0204, 0205, 030201-030204, 030501, 030503, 0307, 0308, 0311, 0318, 0629
5⁵	220 bp	515 bp	3, 7, 8	030101-030103, 0304, 0309, 0310, 0313, 0314, 0316, 0319
6⁵	220 bp	515 bp	2, 8, 9, 3	020101-0205, 030201-030204, 030302-030303, 0306-0308, 0311, 0312, 0315, 0318, 0320
7^{5,6}	135 bp	515 bp	7, 8, 9, 3	030101-030204, 030302-0320
8⁵	195 bp	430 bp	4	0401, 0402

¹Alleles 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 DQ low resolution typings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 bp or more. Size differences shorter than 20 bp are not given. For high resolution SSP kits the 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 band may sometimes be observed. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

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 tubes, or a band of 515 base pairs, for some tubes.

Tube 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 DQ low resolution typing.

In addition, tubes number 4, 5, 6 and 7 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

PLEASE NOTE: All the SSP kits, except the B*37, B*41, B*42, B*46, B*47, B*48, B*49, B*50, B*53, B*67, B*78, B*81 and B*82 kits and the Cw*01, Cw*02, Cw*08, Cw*12, Cw*14, Cw*15, Cw*16, Cw*17 and Cw*18 kits, from *Olerup* SSP AB can be uniquely identified by the number of tubes and the kit-specific pattern of the two differently sized control bands.

In the presence of a specific amplification the intensity of the control band often decreases.

³The serological reactivity of the DQB1*0505, DQB1*0606 to 0608 alleles, the DQB1*0610 to 0630, the DQB1*030202-030204, DQB1*030303 and the DQB1*030502 to 0320 alleles is not known. In this table we have inferred the serological grouping from the naming of the sequence-defined allele.

⁴For several DQB1 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 5'- and 3'-ends of the second exon of the DQB1 gene are conserved within allelic groups.

⁵These primer mixes may yield somewhat less intense specific PCR fragments than the other DQ low resolution primer mixes.

⁶This primer mix may give rise to nonspecific amplifications.

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	Approx. size of spec. PCR product ¹	Size of control band ²	DR serology ³	Amplified HLA-DRB ⁴ alleles
9	205 bp	515 bp	1	010101-010204, 0104-0116
10	195 bp	430 bp	103	0103
11	215, 260 bp	430 bp	15	150101-1522
12	210 bp	430 bp	16	160101-160502, 1607-1611
13	220 bp	430 bp	3, 17, 18, 11	030101-0335, 1107, 1153
14^{5,6}	80, 210 bp	430 bp	3, 17, 11, 13, 14	030101-030104, 0304-0306, 0308-0316, 0318-0320, 0322, 0323, 0325, 0326, 0328, 0330, 0331, 0333, 110201-1103, 111101-111102, 111401-111402, 1116, 1120, 1121, 1136, 1140, 1141, 1148, 1159, 130101-1304, 1308, 1310, 1315-1317, 1319, 1320, 1322-1324, 1327-1329, 1331-1341, 1343, 1345, 1348, 1351-1354, 1357, 1359, 1361, 1363-1366, 1368-1379, 1416, 1419, 1421
15^{5,6}	80, 210 bp	430 bp	3, 18, 11, 13, 14	030201-0303, 0327, 0329, 1126, 1134, 1315, 1319, 1326, 1344, 1353, 1357, 1402-140302, 1406, 1409, 1412, 1413, 1417-1421, 1424, 1427, 1429, 1430, 1433, 1440, 1441, 1447-1449, 1451, 1463
16^{5,6}	100, 175 bp	430 bp	4	040101-0464
17	205, 230 bp	430 bp	7,	070101-070102, 0703-

			13, 14	0712, 1317, 1450
18	165, 215, 250 bp	515 bp	8, 12, 14	080101-080203, 080302- 0819, 0821-0832, 1204, 1411, 1415
19⁵	85, 130, 180 bp	430 bp	3, 9, 11	0308, 090102-0906, 1107, 1153
20 21^{5,8}	205 bp 100, 170 bp	430 bp 430 bp	10 11, 3, 4, 8	100101-100102 0308, 0415, 0461, 0831, 110101-1160, 1162
22⁵	85, 105 bp	430 bp	12, 8	0832, 120101-120202, 120302- 1215
23	210 bp	430 bp	13, 8, 11, 14	0820, 110101-110403, 110601- 110602, 110801-111202, 111401-1116, 1118-1121, 1123-1125, 112701-1133, 1135-1151, 115401- 115402, 1156-1160, 1162, 130101-1308, 1310-1316, 1318-1343, 1345-1379, 140301-140302, 1412, 1416, 1419, 1421, 1422, 1425, 1427, 1440, 1453, 1463
24⁶	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, 1158-1160, 1162, 120201-120202, 1213, 1215, 130101-130201, 1304- 130502, 130701-1309, 1311, 131401-1324, 1326- 1329, 1331, 1332, 1334- 1336, 1338-1343, 1345-

				1355, 1357, 1359, 1361- 1365, 1367-1379, 1415, 1416, 1422, 1424, 1425, 1427, 1437, 1453
25	175 bp	430 bp	3, 13, 14, 8	030101-0307, 0309, 0311- 0333, 0335, 0820, 130101-1316, 1318-1342, 1344, 1346-1366, 1368- 1379, 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
26⁵⁻⁷	100, 140 bp	430 bp	14, 4, 8,13	0462, 0808, 1345, 140101-140102, 1404, 140701-140702, 1410, 1416, 1422, 1425, 1426, 1428, 1431, 1432, 1435, 1437-1439, 1449, 1450, 1453-1455, 1457, 1458, 1460-1462, 1466
27⁵⁻⁹	110, 135, 170 bp	515 bp	14, 3, 9, 11, 12, 13	0308, 0310, 090102-0902, 0904-0906, 1109, 1110, 111202, 1113, 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, 1464-1466
28⁵⁻⁷	110, 175, 225 bp	430 bp	14, 3, 8, 11, 13, 15, 16	0310, 0809, 0820, 0821, 0832, 1113, 1117, 1123, 1125, 1131, 1145, 1152, 1155, 1313, 1318, 1343, 1345, 1347, 1355, 140101-140103, 140301-

				140503, 140701-1408, 1410-1412, 1414-1416, 1418, 1422-142302, 1425- 1428, 1431, 1432, 1434- 1436, 1438-1440, 1442- 1445, 1449, 1450, 1453- 1466, 1521 ^{weakly} , 1604 ^{weakly}
29	230 bp	430 bp	52	DRB3*01010201-0111, 0201-0222, 030101-0303
30¹⁰	215 bp	430 bp	53	DRB4*010101-0107
31	260 bp	430 bp	51	DRB5*010101-0113, 0202- 0205

¹Alleles 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 typings. When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 bp or more. Size differences shorter than 20 bp are not given. For high resolution SSP kits the 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 tubes 11, 26, 27 and 28.

PCR fragments longer than the control band may sometimes be observed. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

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 tubes, or a band of 515 base pairs, for some tubes.

Tube number 9 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, tubes number 18 and 27 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

PLEASE NOTE: All the SSP kits, except the B*37, B*41, B*42, B*46, B*47, B*48, B*49, B*50, B*53, B*67, B*78, B*81 and B*82 kits and the Cw*01, Cw*02, Cw*08, Cw*12, Cw*14, Cw*15, Cw*16, Cw*17 and Cw*18 kits, from *Olerup* SSP AB can be uniquely identified by the number of tubes and the kit-specific pattern of the two differently sized control bands.

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 used the information published in *Tissue Antigens* 1999: **54**: 409-437, also available at the www.worldmarrow.org web site and 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 nucleotide sequences of the DRB1*0334, *110404, *1161, *1163 and *1377 are not yet retrievable.

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

Lot No.: **X80**

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⁶Individual alleles can give rise to two differently sized specific PCR fragments in these primer mixes.

⁷These primer mixes may give rise to nonspecific amplifications.

⁸Primer mix 21 may give rise to primer oligomer formation.

⁹Primer mix 27 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 pair in tube No. 30, whereas the DRB4*0201N and DRB4*0301N null alleles are not amplified by this primer pair.

INTERPRETATION TABLE									
DQ low resolution SSP typing									
Amplification patterns of the DQB1*0501 to DQB1*0402 alleles									
		Tube							
		1	2	3	4	5	6	7	8
Length of spec.		220	220	205	130	220	220	135	195
PCR product			270		145				
Length of int.		515	430	430	515	515	515	515	430
pos. control¹									
5'-primer(s)²		26	9	30	21	26	26	55	26
		5'-g gg ^{3'}	5'-g TT ^{3'}	5'-A Ag ^{3'}	5'-ACC ^{3'}	5'-T TA ^{3'}	26	5'-g CC ^{3'}	5'-g gg ^{3'}
			26		26			55	26
			5'-T TA ^{3'}		5'-T CT ^{3'}			5'-g CA ^{3'}	5'-g gg ^{3'}
			26						
			5'-T CT ^{3'}						
3'-primer(s)³		87	86	86	57	86	86	86	77
		5'-g gT ^{3'}	5'-A Cg ^{3'}	5'-g CT ^{3'}	5'-C gg ^{3'}	5'-g CT ^{3'}	5'-g CT ^{3'}	5'-g CT ^{3'}	5'-AC g ^{3'}
			86						
			5'-A CC ^{3'}						
Tube No.		1	2	3	4	5	6	7	8
DQB1 allele⁴	ser.⁵								
*050101-0505	5	+							
*060101-0628, 0630	6		+						
*0629	6		+		+				
*020101-0202, 0204, 0205	2			+	+		+		
*0203	2			+			+		
*030101-030103, 0304, 0309, 0310, 0313, 0314, 0316, 0319	3, 7, 8					+		+	
*030201-030204, 0307, 0308, 0311, 0318	3, 8				+		+	+	
*030302-030303, 0306, 0312, 0315, 0320	3, 9						+	+	
*030501, 030503	3, 8				+			+	
*030502, 030504, 0317								+	
*0401, 0402	4								+
DQB1 allele⁴	ser.⁵								
Tube No.		1	2	3	4	5	6	7	8

Lot No.: **X80**

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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 tubes, or a band of 515 base pairs, for some tubes. Tube 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 DQ low resolution typing. In addition, tubes number 4, 5, 6 and 7 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

PLEASE NOTE: All the SSP kits, except the B*37, B*41, B*42, B*46, B*47, B*48, B*49, B*50, B*53, B*67, B*78, B*81 and B*82 kits and the Cw*01, Cw*02, Cw*08, Cw*12, Cw*14, Cw*15, Cw*16, Cw*17 and Cw*18 kits, from *Olerup* SSP AB can be uniquely identified by the number of tubes and the kit-specific pattern of the two differently sized control bands.

²The codon, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given. Codon numbering as in *Tissue Antigens* 1998, **51:II**, 467-507. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.

³The codon, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Codon numbering as in *Tissue Antigens* 1998, **51:II**, 467-507. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.

⁴The sequence of the DQB1*030301 allele has been shown to be identical to DQB1*030302.

⁵The serological reactivity of the DQB1*0505, DQB1*0606 to 0608 alleles, the DQB1*0610 to 0630, the DQB1*030202-030204, DQB1*030303 and the DQB1*030502 to 0320 alleles is not known. In this table we have inferred the serological grouping from the naming of the sequence-defined allele.

INTERPRETATION TABLE													
DR low resolution SSP typing													
Amplification patterns of the DRB1*0101 to DRB1*1001 alleles													
		Tube											
		9	10	11	12	13	14	15	16	17	18	19	20
Length of spec.		205	195	215	210	220	80	80	100	205	165	85	205
PCR product				260			210	210	175	230	215	130	
											250	180	
Length of int.		515	430	430	430	430	430	430	430	430	515	430	430
pos. control ¹													
5'-primer(s) ²		14	14	13	13	13	13	13	13	14	16	26	31
		5'-gAA ³	5'-gAA ³	5'-Agg ³	5'-Agg ³	5'-g TC ³	5'-g TC ³	5'-g TC ³	5'-A TA ³	5'-AT A ³	5'-gT T ³	5'-TAT ³	5'-gC g ³
							16		13	14	16	58	
							5'-gT T ³		5'-A CA ³	5'-AT A ³	5'-gT T ³	5'-gAg ³	
									13	16			
									5'-A CC ³	5'-gT T ³			
3'-primer(s) ³		67	67	71	67	73	26	28	33	71	58	57	86
		5'-gAg ³	5'-gAT ³	5'-CgC ³	5'-gAA ³	5'-g gC ³	5'-g gT ³	5'-CT C ³	5'-gTg ³	5'-CTC ³	5'-C CT ³	5'-C gA ³	5'-C AC ³
		71		86	67	73	71	70	58	73	74	73	
		5'-gC g ³		5'-C CA ³	5'-gAg ³	5'-g gC ³	5'-g CT ³	5'-CTg ³	5'-C gg ³	5'-g gC ³	5'-CAg ³	5'-g gC ³	
					72	73				77	86	78	
					5'-gC g ³	5'-T gC ³				5'-A AT ³	5'-C AC ³	5'-CAC ³	
										78			
										5'-CAC ³			
Tube No.	DR	9	10	11	12	13	14	15	16	17	18	19	20
DRB1 allele ⁴	ser ⁵												
*010101-010204, 0104-0116	1	+											
*0103	103		+										
*030101-030104, 0304-0306, 0309, 0311-0316, 0318-0320, 0322, 0323, 0325, 0326, 0328, 0330, 0331, 0333	17					+	+						
*030201-0303, 0327, 0329	18					+		+					
*0307, 0317, 0321, 0324, 0332, 0335	-					+							
*0308	-					+	+					+	
Tube No.	DR	9	10	11	12	13	14	15	16	17	18	19	20

INTERPRETATION TABLE												
DR low resolution SSP typing												
Amplification patterns of the DRB1*0101 to DRB1*1001 alleles												
Tube												
21	22	23	24	25	26	27	28	29	30	31		
100	85	210	195	175	100	110	110	230	215	260		Length of spec.
170	105		210		140	135	175					PCR product
						170	225					
430	430	430	430	430	430	515	430	430	430	430		Length of int.
												pos. control ¹
13	16	10	10	13	37	26	13	10	28	13		5'-primer(s) ²
5'-g TC ^{3'}	5'-gT T ^{3'}	5'-g CT ^{3'}	5'-g CT ^{3'}	5'-g TC ^{3'}	5'-g TA ^{3'}	5'-g TA ^{3'}	5'-g TC ^{3'}	5'-g CT ^{3'}	5'-g AT ^{3'}	5'-g TA ^{3'}		
13		13	13		37	34	34	10		13		
5'-A CA ^{3'}		5'-g TC ^{3'}	5'-g TC ^{3'}		5'-g TT ^{3'}	5'-CAg ^{3'}	5'-CAg ^{3'}	5'-g CT ^{3'}		5'-g TA ^{3'}		
16			16									
5'-gT C ^{3'}			5'-gT T ^{3'}									
38			16									
5'-C gT ^{3'}			5'-gT C ^{3'}									
58	30	70	67	58	57	57	57	73	87	87		3'-primer(s) ³
5'-C CT ^{3'}	5'-gTg ^{3'}	5'-gTC ^{3'}	5'-gAA ^{3'}	5'-C gg ^{3'}	5'-C Ag ^{3'}	5'-C Ag ^{3'}	5'-C Ag ^{3'}	5'-g gC ^{3'}	5'-CTC ^{3'}	5'-CTC ^{3'}		
58	38	71	67	58	71	58	60	73				
5'-C CT ^{3'}	5'-CAg ^{3'}	5'-g CT ^{3'}	5'-gAA ^{3'}	5'-C Ag ^{3'}	5'-CgC ^{3'}	5'-C CT ^{3'}	5'-gTg ^{3'}	5'-g gC ^{3'}				
			71			70	70					
			5'-CTC ^{3'}			5'-CTg ^{3'}	5'-T CC ^{3'}					
			71			70	74					
			5'-CgC ^{3'}			5'-T CC ^{3'}	5'-CAg ^{3'}					
21	22	23	24	25	26	27	28	29	30	31	DR	Tube No.
											ser ⁵	DRB1 allele ⁴
											1	*010101-010204, 0104-0116
											103	*0103
				+							17	*030101-030104, 0304-0306, 0309, 0311-0316, 0318-0320, 0322, 0323, 0325, 0326, 0328, 0330, 0331, 0333
				+							18	*030201-0303, 0327, 0329
				+							-	*0307, 0317, 0321, 0324, 0332, 0335
+						+					-	*0308
21	22	23	24	25	26	27	28	29	30	31	DR	Tube No.

Lot No.: **X80**

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Length of spec.		205	195	215	210	220	80	80	100	205	165	85	205
PCR product				260			210	210	175	230	215	130	
											250	180	
Tube No.	DR	9	10	11	12	13	14	15	16	17	18	19	20
*0310	-					+	+						
*040101-0414, 0416-0460, 0463, 0464	4								+				
*0415, 0461	4								+				
*0462	-								+				
*070101-0712	7									+			
*080101-080203, 080401-0807, 0811, 0816, 0817, 0822, 0824, 0826, 0828	8										+		
*080302, 0810, 0812-0815, 0818, 0819, 0823, 0825, 0827, 0829, 0830	8										+		
*0808	-										+		
*0809, 0821, 1415	8										+		
*0820, 1318, 1347, 1355	8,13												
*0831	-										+		
*0832	-										+		
*090102-0902, 0904-0906	9											+	
*0903	9											+	
*100101-100102	10												+
*110101-110106, 110401-110403, 110601-110602, 111201, 1115, 1124, 112701-1130, 1132, 1133, 1135, 1137-1139, 1143, 1144, 1147, 1149-1151, 115401-115402, 1156, 1160, 1162	11												
*110201-1103, 111101-111102, 111401-111402, 1121, 1136, 1141, 1148	11						+						
*1105	11												
*1107, 1153	-					+						+	
Tube No.	DR	9	10	11	12	13	14	15	16	17	18	19	20

100	85	210	195	175	100	110	110	230	215	260		Length of spec.
170	105		210		140	135	175					PCR product
						170	225					
21	22	23	24	25	26	27	28	29	30	31	DR	Tube No.
						+	+				-	*0310
											4	*040101-0414, 0416-0460, 0463, 0464
+											4	*0415, 0461
					+						-	*0462
											7	*070101-0712
			+								8	*080101-080203, 080401-0807, 0811, 0816, 0817, 0822, 0824, 0826, 0828
											8	*080302, 0810, 0812-0815, 0818, 0819, 0823, 0825, 0827, 0829, 0830
			+		+						-	*0808
			+				+				8	*0809, 0821, 1415
		+	+	+			+				8,13	*0820, 1318, 1347, 1355
+			+								-	*0831
	+						+				-	*0832
						+					9	*090102-0902, 0904-0906
											9	*0903
											10	*100101-100102
+		+	+								11	*110101-110106, 110401-110403, 110601-110602, 111201, 1115, 1124, 112701-1130, 1132, 1133, 1135, 1137-1139, 1143, 1144, 1147, 1149-1151, 115401-115402, 1156, 1160, 1162
+		+	+								11	*110201-1103, 111101-111102, 111401-111402, 1121, 1136, 1141, 1148
+			+								11	*1105
+											-	*1107, 1153
21	22	23	24	25	26	27	28	29	30	31	DR	Tube No.

Lot No.: **X80**

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Length of spec.		205	195	215	210	220	80	80	100	205	165	85	205
PCR product				260			210	210	175	230	215	130	
											250	180	
Tube No.	DR	9	10	11	12	13	14	15	16	17	18	19	20
*110801-110802, 1118-111902, 1142, 1157	11												
*1109, 1110, 111202, 1146, 1158	11												
*1113, 1117, 1152	11												
*1116, 1120, 1140, 1159	-						+						
*1122	-												
*1123, 1125	11												
*1126, 1134	11							+					
*1131, 1145	-												
*1155	-												
*120101-120102, 120302, 1205-1212, 1214	12												
*120201-120202, 1213, 1215	12												
*1204	-										+		
*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, 1379	13						+						
*130202, 130301-130302, 1310, 1333, 1337, 1366	13						+						
*130501-130502, 130701-130702, 1311, 131401-131402, 1321, 1342, 1346, 1349, 1350, 1362	13												
*1306, 1312, 1325, 1330, 1356, 1358, 1360	13												
*1309	13												
*1313	-												
*1315, 1319, 1353, 1357	-						+	+					
Tube No.	DR	9	10	11	12	13	14	15	16	17	18	19	20

100	85	210	195	175	100	110	110	230	215	260		Length of spec.
170	105		210		140	135	175					PCR product
						170	225					
21	22	23	24	25	26	27	28	29	30	31	DR	Tube No.
+		+									11	*110801-110802, 1118-111902, 1142, 1157
+		+	+			+					11	*1109, 1110, 111202, 1146, 1158
+						+	+				11	*1113, 1117, 1152
+		+	+			+					-	*1116, 1120, 1140, 1159
+						+					-	*1122
+		+	+				+				11	*1123, 1125
+											11	*1126, 1134
+		+					+				-	*1131, 1145
+			+				+				-	*1155
	+										12	*120101-120102, 120302, 1205-1212, 1214
	+		+								12	*120201-120202, 1213, 1215
	+					+					-	*1204
		+	+	+							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, 1379
		+		+							13	*130202, 130301-130302, 1310, 1333, 1337, 1366
		+	+	+							13	*130501-130502, 130701-130702, 1311, 131401-131402, 1321, 1342, 1346, 1349, 1350, 1362
		+		+							13	*1306, 1312, 1325, 1330, 1356, 1358, 1360
			+	+							13	*1309
		+		+			+				-	*1313
		+	+	+							-	*1315, 1319, 1353, 1357
21	22	23	24	25	26	27	28	29	30	31	DR	Tube No.

Length of spec.		205	195	215	210	220	80	80	100	205	165	85	205
PCR product				260			210	210	175	230	215	130	
											250	180	
Tube No.	DR	9	10	11	12	13	14	15	16	17	18	19	20
*1317	13						+			+			
*1326	-							+					
*1343	-						+						
*1344	-							+					
*1345	-						+						
*1367	-												
*140101-140102, 1404, 140701-140702, 1410, 1426, 1428, 1431, 1432, 1435, 1438, 1439, 1454, 1455, 1457, 1460-1462, 1466	14												
*140103, 1408, 142302, 1434	-												
*1402, 1406, 1409, 1413, 1417, 1420, 1429, 1430, 1433, 1441, 1447, 1448, 1451	14							+					
*140301-140302, 1412, 1440, 1463	14							+					
*140501-140503, 1414, 142301, 1436, 1443- 1445, 1456, 1459, 1464, 1465	14												
*1411	-										+		
*1416	6						+						
*1418	-							+					
*1419, 1421	-						+	+					
*1422	-												
*1424	-							+					
*1425, 1453	-												
*1427	14							+					
*1437	-												
*1442													
*1446, 1452	-												
*1449	-							+					
*1450	-									+			
*1458	-												
Tube No.	DR	9	10	11	12	13	14	15	16	17	18	19	20

100	85	210	195	175	100	110	110	230	215	260		Length of spec.
170	105		210		140	135	175					PCR product
						170	225					
21	22	23	24	25	26	27	28	29	30	31	DR	Tube No.
			+								13	*1317
		+	+	+							-	*1326
		+	+			+	+				-	*1343
				+							-	*1344
		+	+		+		+				-	*1345
		+	+								-	*1367
					+	+	+				14	*140101-140102, 1404, 140701-140702, 1410, 1426, 1428, 1431, 1432, 1435, 1438, 1439, 1454, 1455, 1457, 1460-1462, 1466
						+	+				-	*140103, 1408, 142302, 1434
				+		+					14	*1402, 1406, 1409, 1413, 1417, 1420, 1429, 1430, 1433, 1441, 1447, 1448, 1451
		+		+			+				14	*140301-140302, 1412, 1440, 1463
				+		+	+				14	*140501-140503, 1414, 142301, 1436, 1443- 1445, 1456, 1459, 1464, 1465
						+	+				-	*1411
		+	+		+	+	+				6	*1416
				+		+	+				-	*1418
		+		+		+					-	*1419, 1421
		+	+		+	+	+				-	*1422
			+	+							-	*1424
		+	+		+		+				-	*1425, 1453
		+	+	+			+				14	*1427
			+	+	+						-	*1437
				+			+					*1442
						+					-	*1446, 1452
					+	+	+				-	*1449
					+	+	+				-	*1450
					+		+				-	*1458
21	22	23	24	25	26	27	28	29	30	31	DR	Tube No.

Lot No.: **X80**

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Length of spec.		205	195	215	210	220	80	80	100	205	165	85	205
PCR product				260			210	210	175	230	215	130	
											250	180	
Tube No.	DR	9	10	11	12	13	14	15	16	17	18	19	20
*150101-1520, 1522	15			+									
*1521	-			+									
*160101-1603, 160501-160502, 1607-1611	16				+								
*1604	16				+								
Tube No.		1	2	3	4	5	6	7	8	9	10	11	12
DRB1 allele ⁴	ser ⁵												
DRB3*01010201-0111, 0201-0222, 030101-0303	52												
DRB4*010101-0107	53												
DRB5*010101-0113, 0202-0205	51												
Tube No.	DR	9	10	11	12	13	14	15	16	17	18	19	20

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 tubes, or a band of 515 base pairs, for some tubes. Tube number 9 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, tubes number 18 and 27 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

PLEASE NOTE: All the SSP kits, except the B*37, B*41, B*42, B*46, B*47, B*48, B*49, B*50, B*53, B*67, B*78, B*81 and B*82 kits and the Cw*01, Cw*02, Cw*08, Cw*12, Cw*14, Cw*15, Cw*16, Cw*17 and Cw*18 kits, from *Olerup* SSP AB can be uniquely identified by the number of tubes and the kit-specific pattern of the two differently sized control bands.

²The codon, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given. Codon numbering as in *Tissue Antigens* 1998, **51:II**, 467-507. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.

³The codon, in the 2nd exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Codon numbering as in *Tissue Antigens* 1998, **51:II**, 467-507. The sequence of the 3 terminal nucleotides of the primer is given. Empty spaces indicate codon boundaries.

Lot No.: **X80**

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100	85	210	195	175	100	110	110	230	215	260		Length of spec.
170	105		210		140	135	175					PCR product
						170	225					
21	22	23	24	25	26	27	28	29	30	31	DR	Tube No.
											15	*150101-1520, 1522
							W				-	*1521
											16	*160101-1603, 160501-160502, 1607-1611
							W				16	*1604
13	14	15	16	17	18	19	20	21	22	23		Tube No.
											ser ⁵	DRB1 allele ⁴
								+			52	DRB3*01010201-0111, 0201-0222, 030101-0303
									+		53	DRB4*010101-0107
										+	51	DRB5*010101-0113, 0202-0205
21	22	23	24	25	26	27	28	29	30	31	DR	Tube No.

⁴The nucleotide sequences of the DRB1*0334, *110404, *1161, *1163 and *1377 are not yet retrievable.

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.

⁵The serological reactivity of all DRB alleles is not known. In this table we used the information published in *Tissue Antigens* 1999: **54**: 409-437, also available at the www.worldmarrow.org web site and also inferred the serological grouping from the naming of the sequence-defined allele.

'w', may be weakly amplified.

'ser', serological HLA specificity.

CELL LINE VALIDATION SHEET									
DQ low resolution primer set									
						Tube			
						1	2	3	4
					Production No.	200734101	200734102	200734103	200734104
						200734105	200734106	200734107	200734108
	cell line	DQB1							
1	9001 SA	*0501				+	-	-	-
2	9280 LK707	*0601	*0202			-	+	+	+
3	9011 E4181324	*0601				-	+	-	-
4	9275 GU373	*0201				-	-	+	+
5	9009 KAS011	*0502				+	-	-	-
6	9353 SM	*0302	*0601			-	+	-	+
7	9020 QBL	*0201				-	-	+	+
8	9007 DEM	*0302	*0502			+	-	-	+
9	9026 YAR	*0302				-	-	-	+
10	9107 LKT3	*0401				-	-	-	-
11	9051 PITOUT	*0202				-	-	+	+
12	9052 DBB	*0303				-	-	-	-
13	9067 BTB	*0402				-	-	-	-
14	9071 OLGA	*0402				-	-	-	-
15	9075 DKB	*0303				-	-	-	-
16	9037 SWEIG007	*0301				-	-	-	-
17	9008 WILJON	*0602	*0603			-	+	-	-
18	9257 32367	*0602	*0202			-	+	+	+
19	9038 BM16	*0301				-	-	-	-
20	9059 SLE005	*0604				-	+	-	-
21	9064 AMALA	*0301				-	-	-	-
22	9056 KOSE	*0503	*0604			+	+	-	-
23	9124 IHL	*0503	*0601			+	+	-	-
24	9035 JBUSH	*0301				-	-	-	-
25	9049 IBW9	*0202				-	-	+	+
26	9285 WT49	*0201				-	-	+	+
27	9191 CH1007	*0401	*0501			+	-	-	-
28	9320 BEL5GB	*0202	*0301			-	-	+	+
29	9050 MOU	*0202				-	-	+	+
30	9021 RSH	*0402				-	-	-	-
31	9019 DUCAF	*0201				-	-	+	+
32	9297 HAG	*0301				-	-	-	-
33	9098 MT14B	*0302				-	-	-	-
34	9104 DHIF	*0301				-	-	-	-
35	9302 SSTO	*0305				-	-	-	-
36	9024 KT17	*0302				-	-	-	-
37	9065 HHKB	*0603				-	+	-	-
38	9099 LZL	*0301				-	-	-	-
39	9315 CML	*0201	*0301			-	-	+	+
40	9134 WHONP199	*0202	*0303			-	-	+	+
41	9055 H0301	*0609				-	+	-	-
42	9066 TAB089	*0601				-	+	-	-
43	9076 T7526	*0303				-	-	-	-
44	9057 TEM	*0503				+	-	-	-
45	9239 SHJO	*0202				-	-	+	+
46	9013 SCHU	*0602				-	+	-	-
47	9045 TUBO	*0301				-	-	-	-
48	9303 TER-ND	*0501				+	-	-	-

CELL LINE VALIDATION SHEET																								
DR low resolution primer set																								
					Tube																			
					9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24				
				Prod. No.:	200734001	200734002	200734003	200734004	200734005	200734006	200734007	200734008	200734009	200734010	200734011	200734012	200734013	200734014	200734015	200734016				
	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												
					Tube							
						25	26	27	28	29	30	31
					Prod. No.:	200734017	200734018	200734019	200734020	200734021	200734022	200734023
	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™ DQ-DR SSP Combi Tray

Product number: 101.704-48/12 – licensed for PCR
101.704-48u/12u – not licensed for PCR
Lot number: X80
Expiry date: 2009-May-01
Number of tests: 48 tests – Product No. 101.704-48
12 tests – Product No. 101.704-12
Number of tubes per test: 31 + 1

Tube specifications:

Tube No.	Production No.
1	2007-341-01
2	2007-341-02
3	2007-341-03
4	2007-341-04
5	2007-341-05
6	2007-341-06
7	2007-341-07
8	2007-341-08

Tube No.	Production No.	Tube No.	Production No.	Tube No.	Production No.
9	2007-340-01	17	2007-340-09	25	2007-340-17
10	2007-340-02	18	2007-340-10	26	2007-340-41
11	2007-340-03	19	2007-340-11	27	2007-340-19
12	2007-340-04	20	2007-340-12	28	2007-340-20
13	2007-340-05	21	2007-340-13	29	2007-340-21
14	2007-340-06	22	2007-340-14	30	2007-340-22
15	2007-340-07	23	2007-340-15	31	2007-340-23
16	2007-340-08	24	2007-340-24		

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

The reactivities of one additional 3'-primer in primer solution 11, one additional 3'-primer in primer solution 12, one additional 5'-primer in primer solution 14, one additional 3'-primer in primer solution 17, one additional primer pair in primer solutions 16, 17 and 19, two additional 5'-primers in primer solution 21, one additional primer pair in primer solution 26, one additional 3'-primer in primer solution 27 and one additional 3'-primer in primer solution 28 were tested by separately adding another 5'-primer or 3'-primer. One of the 5'-primers in primer solution 18 and one of the 5'-primers in primer solution 21 were not possible to test.

The negative control primer pairs, **Production No. 2006-148-01**, can detect contamination with PCR products diluted 10^{-7} .

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

Date of approval: 2007-July-12

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: Olerup SSP™ DQ-DR SSP Combi Tray
Product number: 101.704-48/12, 101.704-48u/12u
Lot number: X80

Intended use: DQB1 and DRB1 low resolution histocompatibility testing

Manufacturer: Olerup SSP AB
Hasselstigen 1
SE-133 33 Saltsjöbaden, Sweden
Phone: +46-8-717 88 27
Fax: +46-8-717 88 18

We, Olerup SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2000 and ISO 13485:2004, 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
2007-July-12

Olle Olerup
Managing Director

WARRANTY

Olerup SSP AB warrants its products to the original purchaser against defects in materials and workmanship under normal use and application. *Olerup* SSP AB's sole obligation under this warranty shall be to replace, at no charge, any product that does not meet the performance standards stated on the product specification sheet.

This warranty applies only to products that have been handled and stored in accordance with *Olerup* SSP AB's recommendations, and does not apply to products that have been the subject of alternation, misuse, or abuse.

All claims under this warranty must be directed to *Olerup* SSP AB in writing and must be accompanied by a copy of the purchaser's invoice. This warranty is in lieu of all other warranties, expressed or implied, including the warranties of merchantability and fitness for a particular purpose. In no case shall *Olerup* SSP AB be liable for incidental or consequential damages.

This product may not be reformulated, repacked or resold in any form without the written consent of *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

Handle all samples as if capable of transmitting disease. All work should be performed wearing gloves and appropriate protection.

Olerup SSPTM is a trademark of *Olerup* SSP AB.
PCRTM is a trademark of F. Hoffmann-La Roche Ltd.
ARMSTM is a trademark of Zeneca Ltd.

DQ-DR SSP Combi Tray
101.704-48/12 – licensed for PCR
101.704-48u/12u – not licensed for PCR
Lot No.: **X80**

36

www.olerup.com

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E-mail: support-at@olerup.com

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

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Fax: 610-344-7989

E-mail: info.us@olerup.com

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

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