

Olerup SSP™ DRB1*04

Product number: 101.114-24/03 – licensed for PCR
101.114-24u/03u – not licensed for PCR
Lot number: Y08
Expiry date: 2009-July-01
Number of tests: 24 tests – Product No. 101.114-24
3 tests – Product No. 101.114-03
Number of tubes per test: 32
Storage - pre-aliquoted primers: dark at -20°C
- PCR Master Mix: -20°C

This Product Description is only valid for Lot No. Y08.

CHANGES COMPARED TO THE PREVIOUS OLERUP SSP™ DRB1*04 LOT

The DRB1*04 specificity and interpretation tables have been updated for the DRB1 alleles reported since the previous *Olerup SSP™* DRB1*04 lot (Lot No. V86) was made.

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

Tube	5'-primer	3'-primer	rationale
17	-	Modified	Increased specificity of specific primer pair.
19	-	Added	Primer added for the DRB1*0462 allele.
20	-	Added	Primer added for the DRB1*0463 allele.
25	Added	-	Primer added for the DRB1*0464 allele.
29	Added	Modified	Primer added for the DRB1*0464 allele. Increased yield of specific PCR product.
31	Added	Modified	Primer added for the DRB1*0464 allele. Increased yield of specific PCR product.

PRODUCT DESCRIPTION

DRB1*04 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*0401 to DRB1*0464 alleles.

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.

32 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.

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

The 32 well cut PCR plate is marked with 'DRB1*04 Y08'.

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

Three DRB1*01, eleven DRB1*03, five DRB1*08, most DRB1*09, nine DRB1*11, many DRB1*13, thirteen DRB1*14 and one DRB1*15 allele will be amplified by the DRB1*04 subtyping kit besides the DRB1*04 group of alleles. In addition, many DRB3 alleles will be amplified by primer mix 24 and the DRB5*0112 allele weakly by primer mix 29. Thus, the interpretation of DRB1*04 subtypings will be influenced by these DRB alleles.

UNIQUELY IDENTIFIED ALLELES

All the phenotypically different DRB1*04 alleles, i.e. **DRB1*0401 to DRB1*0464**, recognized by the HLA Nomenclature Committee in July 2007¹ will give rise to unique amplification patterns by the primers in the DRB1*04 subtyping kit.

The DRB1*04 subtyping kit cannot distinguish the DRB1*040101 and *040102 alleles, the DRB1*040301 to DRB1*040303 alleles, the DRB1*040501 to DRB1*040504 alleles, the DRB1*040601 and DRB1*040602 alleles and the DRB1*040701 to DRB1*040703 alleles.

²**Nomenclature for factors of the HLA system, 1998.** *Tissue Antigens* 1999; **53**: 407-446.

DRB alleles listed on the IMGT/HLA web page 2007-July-09, release 2.19.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 64 DRB1*04 alleles can be combined in 1 770 homozygous and heterozygous combinations. 299 of these genotypes do not give rise to unique amplification patterns. (The different lengths of the specific PCR products generated by the primers in vials 2, 11, 12, 13, 14, 17, 19 to 23, 25, 27, 29 and 31 were not considered in these calculations.)

++-+---+	-+---+-	----	-----	0402, 0438 = 0437, 0438
+-++-++	++-+---+	-+---+-	-+---+-	0416, 0459 = 0435, 0452
+-++-++	++-+---+	--+---+	-+---+-	0416, 0439 = 0452, 0462
+-++-++	++-+---+	---+---+	-+---+-	0416, 0427 = 0452, 0463
+-++-++	++-+---+	-----	-+---+-	0416, 0451 = 0433, 0452
+-++-++	++-+---+	-----	-+---+-	0401, 0452 = 0403, 0416 = 0416, 0452
+-++-++	++-+---+	-++-+---	-+---+-	0435, 0439 = 0459, 0462
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+-++-++	++-+---+	-++-+---	-+---+-	0433, 0439 = 0451, 0462
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+-++-+-	++-+---	-+---+-	++-+---	0426, 0428 = 0435, 0445
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+-++-++	++-+-+--	-+---+-	-+---+-	0416, 0442 = 0423, 0435
+-++-++	++-+-+--	---+---	-+---+-	0416, 0440 = 0423, 0463
+-++-++	++-+-+--	-----	-+---+-	0416, 0444 = 0423, 0433
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+-++-++	++-+++-	---+---	-+---+-	0433, 0456 = 0438, 0444
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+-++-++	++-+++-	---+---	-----	0438, 0453 = 0438, 0456
+-++-++	++-+++-	-++-+---	-+---+-	0435, 0440 = 0442, 0463

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+--+-++	++-----	-+-----	-+-----	0433, 0442 = 0435, 0444
+--+-++	++-----	-+-----	-+-----	0401, 0442 = 0404, 0435 = 0435, 0442
+--+-++	++-----	---+---+	-+-----	0433, 0440 = 0444, 0463
+--+-++	++-----	---+---+	-+-----	0401, 0440 = 0404, 0463 = 0440, 0463
+--+-++	++-----	-----++	-+-----	0401, 0444 = 0404, 0433 = 0433, 0444
+--+-++	++-----	-+-----	-+-----	0401, 0443 = 0408, 0435 = 0435, 0443
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+--+-++	-++-----	-+-----	-+-----	0401, 0435 = 0435, 0435
+--+-++	-++-----	---+---+	-+-----	0401, 0463 = 0463, 0463
+--+-++	-++-----	-----++	-+-----	0401, 0433 = 0433, 0433
+--+-++	-++-----	-----++	++-+---+	0401, 0464 = 0426, 0464
+--+-++	-++-----	-----++	++-+---+	0401, 0426 = 0426, 0426
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-++-++-++	++-----	---+---+	-+-----	0402, 0429 = 0437, 0448
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-++-++-++	++-----	---+---+	-+-----	0404, 0430 = 0430, 0432
-++-++-++	++-----	-----++	-+-----	0405, 0414 = 0414, 0448
-++-++-++	++-----	---+---+	-+-----	0405, 0430 = 0430, 0430
-++-++-++	++-----	---+---+	-----++	0402, 0458 = 0418, 0437
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-++++-++	++-----	-----++	-+-----	0406, 0410 = 0410, 0449
-++++-++	++-----	-++-----	-+-----	0424, 0459 = 0428, 0439
-++++-++	++-----	-+-----	-+-----	0403, 0428 = 0405, 0459 = 0428, 0459
-++++-++	++-----	-+-----	-+-----	0403, 0424 = 0405, 0439 = 0424, 0439
-++++-++	++-----	+-----	-+-----	0405, 0441 = 0441, 0457
-++++-++	++-----	-----++	-+-----	0403, 0410 = 0410, 0460
-++++-++	++-----	+-----	-+-----	0406, 0447 = 0419, 0441
-++++-++	++-----	-+-----	-+-----	0403, 0443 = 0408, 0459 = 0443, 0459
-++++-++	++-----	+-----	-+-----	0408, 0441 = 0441, 0447
-++++-++	++-----	-+-----	-+-----	0423, 0459 = 0442, 0452
-++++-++	++-----	---+---+	-+-----	0423, 0427 = 0440, 0452
-++++-++	++-----	---+---+	-+-----	0423, 0450 = 0432, 0452
-++++-++	++-----	-----++	-+-----	0423, 0451 = 0444, 0452
-++++-++	++-----	-----++	-+-----	0403, 0423 = 0404, 0452 = 0423, 0452
-++++-++	++-----	-++-----	-+-----	0427, 0442 = 0440, 0459
-++++-++	++-----	-+-----	-+-----	0432, 0459 = 0442, 0450
-++++-++	++-----	-+-----	-+-----	0442, 0451 = 0444, 0459
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-++++-++	++-----	---+---+	-+-----	0427, 0432 = 0440, 0450
-++++-++	++-----	---+---+	-+-----	0427, 0444 = 0440, 0451
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-++++-++	++-----	-----++	-+-----	0432, 0451 = 0444, 0450
-++++-++	++-----	---+---+	-+-----	0403, 0432 = 0404, 0450 = 0432, 0450
-++++-++	++-----	-----++	-+-----	0403, 0444 = 0404, 0451 = 0444, 0451
-++++-++	++-----	+-----	-+-----	0404, 0441 = 0436, 0441
-++-++++	++-----	-----++	++++-++	0411, 0420 = 0417, 0449
-++-++-++	++-----	-----++	++++-++	0406, 0412 = 0412, 0446 = 0412, 0449
-++-++-++	++-----	-----++	++++-++	0406, 0411 = 0411, 0449
-++-++-++	++-----	-----++	++++-++	0407, 0411 = 0417, 0460
-++-++-++	++-----	-----++	++++-++	0403, 0412 = 0412, 0460
-++-++-++	++-----	-----++	++++-++	0403, 0411 = 0411, 0460
-++-++-++	++-----	+-----	-+-----	0406, 0454 = 0446, 0454
-++-++-++	++-----	-----++	-+-----	0406, 0431 = 0431, 0446

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--+--++	++-----	-----	-++-+--	0407, 0449 = 0420, 0460
--+--++	++-----	-----	-+--+-	0403, 0420 = 0406, 0407
--+--+-	++-+-	-----	-+-----	0406, 0418 = 0418, 0446
--+--+-	++-+-	+-----	-+-----	0406, 0425 = 0425, 0446 = 0441, 0446
--+--+-	++-+-	-----	-+-----	0406, 0458 = 0446, 0458
--+--+-	++-+-	-----	-+-----	0406, 0455 = 0446, 0455
--+--+-	++-+-	-----	-+-----	0406, 0446 = 0446, 0446
--+--+-	++-----	-----	-++-----	0403, 0449 = 0406, 0460 = 0449, 0460
--+--+-	++-----	-----	-++-----	0406, 0449 = 0449, 0449
--+-----	++-+-	+-----	-+-----	0431, 0441 = 0441, 0454
--+-----	++-+-	-----	-+-----	0403, 0452 = 0452, 0452
--+-----	++-+-	+-----	-+-----	0425, 0441 = 0441, 0455
--+-----	++-----	-+-----	-+-----	0403, 0459 = 0459, 0459
--+-----	++-----	-+-----	-+-----	0403, 0439 = 0439, 0439
--+-----	++-----	-+-----	-+-----	0403, 0427 = 0427, 0427
--+-----	++-----	-----	-+-----	0403, 0450 = 0450, 0450
--+-----	++-----	-----	-+-----	0403, 0451 = 0451, 0451
--+-----	++-----	-----	-++-----	0403, 0460 = 0460, 0460
---++-++	++-+-	+-----	-+-----	0418, 0457 = 0425, 0448
---++-++	++-+-	-----	-+-----	0418, 0429 = 0448, 0458
---++-++	++-+-	-----	-+-----	0405, 0418 = 0418, 0448 = 0448, 0455
---++-++	++-+-	+-----	-+-----	0425, 0429 = 0457, 0458
---++-++	++-+-	+-----	-+-----	0405, 0425 = 0425, 0457 = 0455, 0457
---++-++	++-+-	-----	-+-----	0405, 0458 = 0429, 0455 = 0429, 0458
---++-++	++-+-	+-----	-+-----	0436, 0448 = 0453, 0457
---++-++	++-+-	-+-----	-+-----	0428, 0453 = 0442, 0448
---++-++	++-+-	-----	-+-----	0405, 0456 = 0429, 0453 = 0429, 0456 = 0448, 0456
---++-++	++-+-	-----	-+-----	0404, 0448 = 0405, 0453 = 0448, 0453
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---++-++	+--+-	-----	-++-----	0405, 0412 = 0412, 0448
---++-+-	++-+-	+-----	-+-----	0405, 0454 = 0431, 0457 = 0454, 0457
---++-+-	++-----	++-----	-+-----	0428, 0447 = 0443, 0457
---++-+-	++-----	+-----	-+-----	0405, 0447 = 0408, 0457 = 0447, 0457
---++-+-	++-----	-+-----	-+-----	0405, 0443 = 0408, 0428 = 0428, 0443
---++-+-	+-----	-----	-+-----	0405, 0448 = 0448, 0448
---++-+-	+-----	+-----	-+-----	0405, 0457 = 0457, 0457
---++-+-	+-----	-+-----	-+-----	0405, 0428 = 0428, 0428
---++-+-	+-----	-+-----	-+-----	0405, 0424 = 0424, 0424
---++-+-	+-----	-----	-+-----	0405, 0429 = 0429, 0429
---++-+-	+-----	-----	++-+-	0405, 0464 = 0445, 0464
---++-+-	+-----	-----	++-+-	0405, 0445 = 0445, 0445
---++-+-	++-+-	-----	-++-----	0404, 0412 = 0410, 0418
---+-++	+++--	+-----	-+-----	0415, 0431 = 0425, 0461
---+-++	+++--	+-----	-+-----	0408, 0415 = 0422, 0447 = 0436, 0461
---+-++	+++--	-+-----	-+-----	0422, 0443 = 0442, 0461
---+-++	+++--	-----	-+-----	0404, 0461 = 0408, 0422 = 0422, 0461
---+-++	++-+-	+-----	-----	0418, 0447 = 0453, 0454
---+-++	++-+-	-----	-+-----	0408, 0418 = 0431, 0453
---+-++	++-+-	++-----	-+-----	0425, 0443 = 0442, 0454
---+-++	++-+-	+-----	-+-----	0404, 0454 = 0408, 0425 = 0431, 0436 = 0447, 0455

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---+---++ ++---+---	+-----	-----+	0425, 0447 = 0436, 0454
---+---++ ++---+---	-+-----	-+-----+	0431, 0442 = 0443, 0455
---+---++ ++---+---	-----	-+-----+	0404, 0431 = 0408, 0455
---+---++ ++-----	++-----	-+-----+	0436, 0443 = 0442, 0447
---+---++ ++-----	+-----	-+-----+	0404, 0447 = 0408, 0436
---+---++ ++-----	-+-----	-+-----+	0404, 0443 = 0408, 0442 = 0442, 0443
---+---+ ++---+---	+-----	-+-----+	0408, 0454 = 0431, 0447
---+---+ ++-----	-+-----	-+-----+	0408, 0443 = 0443, 0443
---+---+ +++-----	+-----	-+-----+	0404, 0415 = 0415, 0422 = 0422, 0436
---+---+ ++-+-----	-----	-+-----+	0404, 0423 = 0423, 0423
---+---+ ++-++--	+-----	-----+	0418, 0436 = 0425, 0453
---+---+ ++-++--	---+---	-----+	0418, 0456 = 0453, 0458 = 0456, 0458
---+---+ ++-++--	-----	-+-----+	0404, 0418 = 0453, 0455
---+---+ ++---+---	+-----	-+-----+	0404, 0425 = 0436, 0455
---+---+ ++---+---	---+---	-----+	0453, 0456 = 0456, 0456
---+---+ ++-----	-+-----	-+-----+	0404, 0442 = 0442, 0442
---+---+ ++-----	---+---	-+-----+	0404, 0440 = 0440, 0440
---+---+ ++-----	-----+---	-+-----+	0404, 0432 = 0432, 0432
---+---+ ++-----	-----+---	-+-----+	0404, 0444 = 0444, 0444
-----++ ++---+---	+-----	-+-----+	0425, 0431 = 0454, 0455

LICENSES

101.114-24/03 – licensed for PCR.

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101.114-24/03 and 101.114-24u/03u

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GUARANTEE

Olerup SSP AB guarantees that the primers in the DRB1*04 subtyping kit 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 DRB1*04 subtyping, add at room temperature in a 0.5 ml tube:

38 x 2 μl = 76 μl DNA (30 ng/μl)

38 x 3 μl = 114 μl PCR Master Mix complete with *Taq* – mix well
before taking your aliquot

38 x 5 μl = 190 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-H₂O mixture into each of the 32 wells of a DRB1*04 subtyping. **Well No. 1 of the 32 well PCR plate is marked with '1'.** Close the 32 well PCR plate with the provided lids.

101.114-24u/03u – not licensed for PCR

For one DRB1*04 subtyping, add at room temperature in a 0.5 ml tube:

38 x 2 μl = 76 μl DNA (30 ng/μl)

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

3.0 μl *Taq* polymerase (5 units/μl)

38 x 5 μl – 3.0 μl = 187 μl dH₂O

Mix well, dispense 10 μl of the DNA-PCR Master Mix-*Taq*-H₂O mixture into each of the 32 wells of a DRB1*04 subtyping. **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 <i>Olerup</i> SSP kits.
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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

DRB1*04 SSP subtyping

Specificities and sizes of the PCR products of the 32 primer mixes used for DRB1*04 SSP subtyping

Primer Mix	Approx. size of spec. PCR product ¹	Size of control band ²	Amplified DRB1*04 alleles ³	Other amplified DRB alleles ⁴
1	215 bp	515 bp	040101-040102, 0409, 0413, 0416, 0421, 0426, 0433, 0435, 0438, 0462, 0463	
2⁸	150, 215 bp	430 bp	0402, 0414, 0430, 0437	1457
3	225 bp	430 bp	040301-040303, 040601-040703, 0411, 0417, 0420, 0427, 0439, 0441, 0446, 0449-0452, 0459, 0460	1410
4	225 bp	515 bp	040101-0402, 0404-040504, 0408-0410, 0413-0416, 0419, 0421, 0423, 0424, 0426, 0428-0430, 0432, 0433, 0435-0438, 0440, 0442-0445, 0447, 0448, 0453, 0456, 0457, 0461-0463	1122, 1457
5	170 bp	430 bp	040501-040504, 0409-0412, 0417, 0424, 0428-0430, 0445, 0448, 0457	
6⁵	115 bp	430 bp	040601-040602, 0419-0421, 0446, 0449	
7	260 bp	430 bp	040101-040102, 040501-040504, 040701-0409, 0414, 0416, 0417, 0419-0421, 0424, 0426, 0428-0431, 0433, 0435, 0438, 0443, 0445, 0447, 0448, 0454, 0457, 0461-0463	1122
8	260 bp	430 bp	0402-0404, 040601-040602, 0410-0413, 0415, 0418, 0422, 0423, 0425, 0427, 0432, 0436, 0437, 0439-0442, 0444, 0446, 0449-0453, 0455, 0456, 0458-0460	1410, 1457
9⁶	215 bp	430 bp	040301-0408, 0410, 0411, 0412 ^{weakly} , 0415, 0417,	1122, 1410

			0418 ^{weakly} , 0419, 0420, 0423, 0424, 0425 ^{weakly} , 0427-0430, 0431 ^{weakly} , 0432, 0436, 0439-0461 040101-0404, 040601- 0408, 0413, 0414, 0416, 0418-0423, 0425, 0426 ^{weakly} , 0427, 0431- 0433, 0435-0444, 0446, 0447, 0449-0456, 0458- 0460, 0463	
10	170 bp	515 bp		
11 ⁹	175, 220 bp	430 bp	0415, 0422, 0461	1122
12 ¹⁰	180, 265 bp	515 bp	0416, 0423, 0452	
13 ¹¹	170, 225 bp	430 bp	0412, 0418, 0425, 0431, 0446, 0454, 0455, 0458	
14 ^{7,12}	200, 220 bp	430 bp	0402, 0412, 0414, 0418, 0438, 0448, 0453, 0456	1457
15 ⁶	215 bp	430 bp	0434	
16 ^{6,7,13}	150 bp	515 bp	040301-040303, 040701- 040703, 0411, 0417, 0427, 0439, 0450 ^{weakly} , 0451, 0452, 0459, 0460	0829, 1153, 115401 ^{weakly} , 115402, 1442 ^{weakly} , 1458 ^{weakly} , DRB3*020205
17 ^{5,14}	110, 190 bp	430 bp	0415, 0425, 0436, 0441, 0447, 0454, 0457	1122
18 ^{5,6}	145 bp	430 bp	0428, 0435, 0442, 0443, 0459	1122
19 ^{5,6,15}	140, 175, 210 bp	515 bp	0424, 0439, 0462	1410, 1457
20 ^{5,16}	100, 175, 255 bp	430 bp	0427, 0440, 0463	
21 ¹⁷	155, 200 bp	515 bp	0429, 0437, 0438, 0456, 0458	
22 ¹⁸	150, 230 bp	430 bp	0430, 0432, 0450	
23 ^{5,19}	120, 240 bp	430 bp	0433, 0444, 0451	
24 ⁵	85 bp	430 bp	040101-040102, 0409, 0416, 0421, 0426, 0433- 0435, 0438, 0462-0464	0110, 0116, 030201-030202, 030501-030502, 0309, 0314, 0317, 0327, 0329, 0335, 1153, 1419, DRB3*01010201 -0111, DRB3*020201- 0203, 0205- 0213, 0215- 0222,

DRB3*0303

25 ^{5,20}	125, 230, 260 bp	430 bp	0426, 0445, 0464	
26	200 bp	515 bp	040101-040102, 040301-0411, 0413, 0416, 0417, 0419-0424, 0426-0433, 0435, 0439-0446, 0448-0452, 0455, 0457, 0459-0463	1410
27 ^{5,6,21}	120, 265 bp	515 bp	0410-0412, 0449, 0460	0312, 0806, 0810, 0812, 0822, 1304, 1332, 1348, 1358, 1375, 1465, 1512
28 ⁵	90 bp	430 bp	0411, 0417	0829
29 ^{5,22}	75, 260 bp	430 bp	040701-040703, 0417, 0420, 0464	090102-0902 ^{weakly} , 0904 ^{weakly} , 0905 ^{weakly} , 140701-140702 ^{weakly} , 1414 ^{weakly} , 1436 ^{weakly} , 1442 ^{weakly} , 1444 ^{weakly} , 1451, 1468,
30 ⁵	80 bp	430 bp	0409	DRB5*0112 ^{weakly} , 130301-1304, 1332, 1333, 1338, 1348, 1365, 1366, 1375
31 ^{5,23}	85, 260 bp	430 bp	0414, 0464	0103, 111101-111102, 111401-111402, 1120, 130201-130302, 1323, 1329, 1331, 1333, 1334, 1336-1339, 1341, 1345, 1363, 1365-1367, 1373, 1374
32 ⁵	115 bp	430 bp	040101-040504, 040701-0418, 0422-0433, 0435-0445, 0447, 0448, 0450-0463	1122

¹ Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of DRB1*04 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 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 3'-primers in primer solutions 9, 18 and 19.

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.

² The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 430 base pairs, for most 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 DRB1*04 subtyping.

In addition, tubes number 4, 10, 12, 16, 19, 21, 26 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.

³ For several DRB alleles only partial second exon nucleotide sequences are available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. We assume that unknown sequences in the first hyperpolymorphic region of the second exon of DRB alleles are conserved within allelic groups and that unknown sequences of codons 87 to 92 are identical with the DRB1*0101 consensus sequence.

⁴ The DRB1*1122, DRB1*1410 and DRB1*1445 alleles carry the DRB1*04 sequence motif in the 1st hyperpolymorphic region of the 2nd exon and will, thus, be amplified by several of the DRB1*04 primer mixes. In addition, three DRB1*01, eleven DRB1*03, five DRB1*08, most DRB1*09, nine DRB1*11, many DRB1*13, thirteen DRB1*14 and one DRB1*15 allele will be amplified by the DRB1*04 subtyping kit. Furthermore, many DRB3 alleles will be amplified by primer mix 24. However, the purpose of primer mix 24 is to be able to distinguish the DRB1*0401, 0404 and DRB1*0408, 0413 genotypes. Non-DRB1*04 alleles will be amplified by primer mixes 2 to 4, 7 to 9, 11, 14, 16 to 18, 24, 26 to 32, but the purpose of these primer mixes is to increase the resolution in DRB1*04 homozygotes.

⁵ Short specific PCR fragments are less intense and not as sharp as longer specific bands.

⁶ Primer mixes 9, 15, 16, 18, 19 and 27 may give rise to nonspecific amplifications.

⁷ Primer mixes 14 and 16 have a tendency of giving rise to primer dimer formation.

⁸ Primer mix 2: Specific PCR fragment of 150 bp in the DRB1*0430 allele. Specific PCR fragment of 215 bp in the DRB1*0402, 0414, 0437 and the DRB1*1457 alleles.

⁹ Primer mix 11: Specific PCR fragment of 175 bp in the DRB1*0415, 0461 and the DRB1*1122 alleles. Specific PCR fragment of 220 bp in the DRB1*0422 allele.

¹⁰ Primer mix 12: Specific PCR fragment of 180 bp in the DRB1*0416 allele. Specific PCR fragment of 265 bp in the DRB1*0423 and 0452 alleles.

¹¹ Primer mix 13: Specific PCR fragment of 170 bp in the DRB1*0446 allele. Specific PCR fragments of 225 bp in the DRB1*0412, 0418, 0425, 0431, 0454, 0455 and 0458 alleles.

¹² Primer mix 14: Specific PCR fragment of 200 bp in the DRB1*0402, 0412, 0414, 0418, 0438, 0453, 0456 and the DRB1*1457 alleles. Specific PCR fragment of 220 bp in the DRB1*0448 allele.

¹³ Primer mix 16 may yield somewhat less specific PCR fragment than the other DRB1*04 primer mixes.

¹⁴ Primer mix 17: Specific PCR fragment of 110 bp in the DRB1*0441 allele. Specific PCR fragment of 190 bp in the DRB1*0415, 0425, 0436, 0447, 0454, 0457 and DRB1*1122 alleles.

¹⁵Primer mix 19: Specific PCR fragment of 140 bp in the DRB1*0439 allele. Specific PCR fragment of 175 bp in the DRB1*0462 allele. Specific PCR fragment of 210 bp in the DRB1*0424 and 1410 alleles.

¹⁶Primer mix 20: Specific PCR fragment of 100 bp in the DRB1*0463 allele. Specific PCR fragment of 175 bp in the DRB1*0440 allele. Specific PCR fragment of 255 bp in the DRB1*0427 allele.

¹⁷Primer mix 21: Specific PCR fragment of 155 bp in the DRB1*0429 allele. Specific PCR fragment of 200 bp in the DRB1*0437, 0438, 0456 and 0458 alleles.

¹⁸Primer mix 22: Specific PCR fragment of 150 bp in the DRB1*0430 allele. Specific PCR fragment of 230 bp in the DRB1*0432 and 0450 alleles.

¹⁹Primer mix 23: Specific PCR fragment of 120 bp in the DRB1*0433 allele. Specific PCR fragment of 240 bp in the DRB1*0444 and 0451 alleles.

²⁰Primer mix 25: Specific PCR fragment of 125 bp in the DRB1*0426 allele. Specific PCR fragment of 230 bp in the DRB1*0445 allele. Specific PCR fragment of 260 bp in the DRB1*0464 allele.

²¹Primer mix 27: Specific PCR fragment of 120 bp in the DRB1*0410, 0411, 0412, 0460 and the DRB1*0312, 0806, 0810, 0812, 0822, 1304, 1332, 1348, 1358, 1375, 1465 and 1512 alleles. Specific PCR fragment of 265 bp in the DRB1*0449 allele.

²²Primer mix 29: Specific PCR fragment of 75 bp in the DRB1*040701-040703, 0417, 0420 and the DRB1*090102-0902^{weakly}, 0904^{weakly}, 0905^{weakly}, 140701-140702^{weakly}, 1414^{weakly}, 1436^{weakly}, 1442^{weakly}, 1444^{weakly}, 1451, 1468 and the

DRB5*0112^{weakly} alleles. Specific PCR fragment of 260 bp in the DRB1*0464 allele.

²³Primer mix 31: Specific PCR fragment of 85 bp in the DRB1*0414 and the DRB1*0103, 111101-111102, 111401-111402, 1120, 130201-130302, 1323, 1329, 1331, 1333, 1334, 1336-1339, 1341, 1345, 1363, 1365-1367, 1373 and 1374 alleles. Specific PCR fragment of 260 bp in the DRB1*0464 allele.

INTERPRETATION TABLE																
DRB1*04 SSP subtyping																
Allele-specific amplification patterns of the DRB1*0401 to *0464 alleles																
	Tube ⁴															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Length of spec.	215	150	225	225	170	115	260	260	215	170	175	180	170	200	215	150
PCR product		215									220	265	225	220		
Length of int.	515	430	430	515	430	430	430	430	430	515	430	515	430	430	430	515
pos. control ¹																
5'-primer(s) ²	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	37
	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-g TA ³
3'-primer(s) ³	71	49	74	74	57	37	86	86	71	57	58	59	56	67	71	74
	5'-CTT ³	5'-C CA ³	5'-C CT ³	5'-C Cg ³	5'-gCT ³	5'-C gg ³	5'-C AC ³	5'-C CA ³	5'-CCT ³	5'-ATC ³	5'-C CT ³	5'-CTg ³	5'-AgC ³	5'-gAT ³	5'-CTT ³	5'-C CT ³
		71							71	57	73	88	74	73		
		5'-CTC ³							5'-CCT ³	5'-ATC ³	5'-g gC ³	5'-AA T ³	5'-CA g ³	5'-ggA ³		
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
DRB1 allele																
*040101-040102	+			+			+			+						
*0402		+		+				+		+				+		
*040301-040303			+					+	+	+						+
*0404				+				+	+	+						
*040501-040504				+	+		+		+							
*040601-040602			+			+		+	+	+						
*040701-040703			+				+		+	+						+
*0408				+			+		+	+						
*0409	+			+	+		+									
*0410				+	+			+	+							
*0411			+		+			+	+							+
*0412					+			+	W				+	+		
*0413	+			+				+		+						
*0414		+		+			+			+				+		
*0415				+				+	+		+					
*0416	+			+			+			+		+				
*0417			+		+		+		+							+
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

INTERPRETATION TABLE																
DRB1*04 SSP subtyping																
Allele-specific amplification patterns of the DRB1*0401 to *0464 alleles																
Tube ⁴																
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
110	145	140	100	155	150	120	85	125	200	120	90	75	80	85	115	Length of spec.
190		175	175	200	230	240		230		265		260		260		PCR product
		210	255					260								
430	430	515	430	515	430	430	430	430	515	515	430	430	430	430	430	Length of int.
																pos. control ¹
13	13	13	13	13	13	13	71	13	13	11	57	13	57	13	13	5'-primer(s) ²
5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-g AA ³	5'-A TA ³	5'-A CA ³	5'-Ag A ³	5'-AgC ³	5'-A TA ³	5'-AgC ³	5'-A TA ³	5'-A CA ³	
								23		57		74		71		
								5'-g CC ³		5'-AgC ³		5'-C gA ³		5'-C gA ³		
								58		63				71		
								5'-AT A ³		5'-CC A ³				5'-C AA ³		
37	47	46	33	51	49	39	86	86	67	86	74	86	71	86	37	3'-primer(s) ³
5'-gTT ³	5'-g gA ³	5'-TA g ³	5'-CgC ³	5'-C CA ³	5'-C CA ³	5'-A gT ³	5'-C AC ³	5'-C AC ³	5'-gAg ³	5'-C CA ³	5'-C CT ³	5'-C AC ³	5'-g CT ³	5'-C AC ³	5'-C gT ³	
60		57	58	67	72	77										
5'-gTT ³		5'-C Ag ³	5'-C gC ³	5'-gAT ³	5'-C CT ³	5'-TA A ³										
67		70	85	67	78	81										
5'-gAA ³		5'-T CC ³	5'-C Ag ³	5'-gAg ³	5'-gTg ³	5'-gTA ³										
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.
																DRB1 allele
							+		+						+	*040101-040102
															+	*0402
									+						+	*040301-040303
									+						+	*0404
									+						+	*040501-040504
									+						+	*040601-040602
									+			+			+	*040701-040703
									+						+	*0408
							+		+				+		+	*0409
									+	+					+	*0410
									+	+	+				+	*0411
										+					+	*0412
									+						+	*0413
														+	+	*0414
+															+	*0415
							+		+						+	*0416
									+		+	+			+	*0417
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.

Length of spec.	215	150	225	225	170	115	260	260	215	170	175	180	170	200	215	150
PCR product		215									220	265	225	220		
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*0418								+	W	+			+	+		
*0419				+		+	+		+	+						
*0420			+			+	+		+	+						
*0421	+			+		+	+			+						
*0422								+		+	+					
*0423				+				+	+	+		+				
*0424				+	+		+		+							
*0425								+	W	+			+			
*0426	+			+			+			W						
*0427			+					+	+	+						+
*0428				+	+		+		+							
*0429				+	+		+		+							
*0430		+		+	+		+		+							
*0431							+		W	+			+			
*0432				+				+	+	+						
*0433	+			+			+			+						
*0434															+	
*0435	+			+			+			+						
*0436				+				+	+	+						
*0437		+		+				+		+						
*0438	+			+			+			+				+		
*0439			+					+	+	+						+
*0440				+				+	+	+						
*0441			+					+	+	+						
*0442				+				+	+	+						
*0443				+			+		+	+						
*0444				+				+	+	+						
*0445				+	+		+		+							
*0446			+			+		+	+	+			+			
*0447				+			+		+	+						
*0448				+	+		+		+					+		
*0449			+			+		+	+	+						
*0450			+					+	+	+						W
*0451			+					+	+	+						+
*0452			+					+	+	+		+				+
*0453				+				+	+	+				+		
*0454							+		+	+			+			
*0455								+	+	+			+			
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

110	145	140	100	155	150	120	85	125	200	120	90	75	80	85	115	Length of spec. PCR product
190		175	175	200	230	240		230		265		260		260		
		210	255					260								
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.
															+	*0418
									+							*0419
									+			+				*0420
							+		+							*0421
									+						+	*0422
									+						+	*0423
		+							+						+	*0424
+															+	*0425
							+	+	+						+	*0426
			+						+						+	*0427
	+								+						+	*0428
				+					+						+	*0429
					+				+						+	*0430
									+						+	*0431
					+				+						+	*0432
						+	+		+						+	*0433
							+									*0434
	+						+		+						+	*0435
+															+	*0436
				+											+	*0437
				+			+								+	*0438
		+							+						+	*0439
			+						+						+	*0440
+									+						+	*0441
	+								+						+	*0442
	+								+						+	*0443
						+			+						+	*0444
								+	+						+	*0445
									+							*0446
+															+	*0447
									+						+	*0448
									+	+						*0449
					+				+						+	*0450
						+			+						+	*0451
									+						+	*0452
															+	*0453
+															+	*0454
									+						+	*0455
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.

Length of spec.	215	150	225	225	170	115	260	260	215	170	175	180	170	200	215	150
PCR product		215									220	265	225	220		
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*0456				+				+	+	+				+		
*0457				+	+		+		+							
*0458								+	+	+			+			
*0459			+					+	+	+						+
*0460			+					+	+	+						+
*0461				+			+		+		+					
*0462	+			+			+									
*0463	+			+			+			+						
*0464																
*0103, 111101- 111102, 111401- 111402, 1120, 130201-130203, 1323, 1329, 1331, 1334, 1336, 1337, 1339, 1341, 1345, 1363, 1367, 1373, 1374																
*0110, 0116, 030201-030202, 030501-030502, 0309, 0314, 0317, 0327, 0329, 0335, 1419																
*0312, 0806, 0810, 0812, 0822, 1358, 1465, 1512																
*0829																+
*090102-0902, 0904, 0905, 140701-140702, 1414, 1436, 1444, 1468																
*1122				+			+		+		+					
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

110	145	140	100	155	150	120	85	125	200	120	90	75	80	85	115	Length of spec. PCR product
190		175	175	200	230	240		230		265		260		260		
		210	255					260								
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.
				+											+	*0456
+									+						+	*0457
				+											+	*0458
	+								+						+	*0459
									+	+					+	*0460
									+						+	*0461
		+					+		+						+	*0462
			+				+		+						+	*0463
							+	+				+		+		*0464
														+		*0103, 111101- 111102, 111401- 111402, 1120, 130201-130203, 1323, 1329, 1331, 1334, 1336, 1337, 1339, 1341, 1345, 1363, 1367, 1373, 1374
							+									*0110, 0116, 030201-030202, 030501-030502, 0309, 0314, 0317, 0327, 0329, 0335, 1419
										+						*0312, 0806, 0810, 0812, 0822, 1358, 1465, 1512
											+					*0829
												W				*090102-0902, 0904, 0905, 140701-140702, 1414, 1436, 1444, 1468
+	+														+	*1122
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.

Length of spec.	215	150	225	225	170	115	260	260	215	170	175	180	170	200	215	150
PCR product		215									220	265	225	220		
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
*1153																+
*115401																W
*115402, 1458																+
*130301- 130302, 1333, 1338, 1365, 1366																
*1304, 1332, 1348, 1375																
*1410			+					+	+							
*1442																W
*1451																
*1457		+		+				+						+		
DRB1 allele																
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
DRB3*01010201- 0111, 020201- 020204, 0203, 0205-0213, 0215 0222, 0303																
DRB3*020205																+
DRB5*0112																
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

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 DRB1*04 subtyping. In addition, tubes number 4, 10, 12, 16, 19, 21, 26 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.

110	145	140	100	155	150	120	85	125	200	120	90	75	80	85	115	Length of spec. PCR product
190		175	175	200	230	240		230		265		260		260		
		210	255					260								
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.
							+									*1153
																*115401
																*115402, 1458
													+	+		*130301- 130302, 1333, 1338, 1365, 1366
										+			+			*1304, 1332, 1348, 1375
		+							+							*1410
												W				*1442
												+				*1451
		+		+												*1457
																DRB1 allele
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.
							+									DRB3*01010201 0111, 020201- 020204, 0203, 0205-0213, 0215 0222, 0303
							+									DRB3*020205
												W				DRB5*0112
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Tube No.

⁴Primer mix 2: Specific PCR fragment of 150 bp in the DRB1*0430 allele. Specific PCR fragment of 215 bp in the DRB1*0402, 0414, 0437 and the DRB1*1457 alleles.

Primer mix 11: Specific PCR fragment of 175 bp in the DRB1*0415, 0461 and the DRB1*1122 alleles. Specific PCR fragment of 220 bp in the DRB1*0422 allele.

Primer mix 12: Specific PCR fragment of 180 bp in the DRB1*0416 allele. Specific PCR fragment of 265 bp in the DRB1*0423 and 0452 alleles.

Primer mix 13: Specific PCR fragment of 170 bp in the DRB1*0446 allele. Specific PCR fragments of 225 bp in the DRB1*0412, 0418, 0425, 0431, 0454, 0455 and 0458 alleles.

Primer mix 14: Specific PCR fragment of 200 bp in the DRB1*0402, 0412, 0414, 0418, 0438, 0453, 0456 and the DRB1*1457 alleles. Specific PCR fragment of 220 bp in the DRB1*0448 allele.

Primer mix 17: Specific PCR fragment of 110 bp in the DRB1*0441 allele. Specific PCR fragment of 190 bp in the DRB1*0415, 0425, 0436, 0447, 0454, 0457 and DRB1*1122 alleles.

Primer mix 19: Specific PCR fragment of 140 bp in the DRB1*0439 allele. Specific PCR fragment of 175 bp in the DRB1*0462 allele. Specific PCR fragment of 210 bp in the DRB1*0424 and 1410 alleles.

Primer mix 20: Specific PCR fragment of 100 bp in the DRB1*0463 allele. Specific PCR fragment of 175 bp in the DRB1*0440 allele. Specific PCR fragment of 255 bp in the DRB1*0427 allele.

Primer mix 21: Specific PCR fragment of 155 bp in the DRB1*0429 allele. Specific PCR fragment of 200 bp in the DRB1*0437, 0438, 0456 and 0458 alleles.

Primer mix 22: Specific PCR fragment of 150 bp in the DRB1*0430 allele. Specific PCR fragment of 230 bp in the DRB1*0432 and 0450 alleles.

Primer mix 23: Specific PCR fragment of 120 bp in the DRB1*0433 allele. Specific PCR fragment of 240 bp in the DRB1*0444 and 0451 alleles.

Primer mix 25: Specific PCR fragment of 125 bp in the DRB1*0426 allele. Specific PCR fragment of 230 bp in the DRB1*0445 allele. Specific PCR fragment of 260 bp in the DRB1*0464 allele.

Primer mix 27: Specific PCR fragment of 120 bp in the DRB1*0410, 0411, 0412, 0460 and the DRB1*0312, 0806, 0810, 0812, 0822, 1304, 1332, 1348, 1358, 1375, 1465 and 1512 alleles. Specific PCR fragment of 265 bp in the DRB1*0449 allele.

Primer mix 29: Specific PCR fragment of 75 bp in the DRB1*040701-040703, 0417, 0420 and the DRB1*090102-0902^{weakly}, 0904^{weakly}, 0905^{weakly}, 140701-140702^{weakly}, 1414^{weakly}, 1436^{weakly}, 1442^{weakly}, 1444^{weakly}, 1451, 1468 and the

DRB5*0112^{weakly} alleles. Specific PCR fragment of 260 bp in the DRB1*0464 allele.

Primer mix 31: Specific PCR fragment of 85 bp in the DRB1*0414 and the DRB1*0103, 111101-111102, 111401-111402, 1120, 130201-130302, 1323, 1329, 1331, 1333, 1334, 1336-1339, 1341, 1345, 1363, 1365-1367, 1373 and 1374 alleles. Specific PCR fragment of 260 bp in the DRB1*0464 allele.

'w', might be weakly amplified.

CELL LINE VALIDATION SHEET																				
DRB1*04 SSP subtyping kit																				
					Tube															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
				Prod. No.	200736201	200736202	200736203	200736204	200736205	200736206	200736207	200736208	200736209	200736210	200736211	200736212	200736213	200736214	200736215	200736216
	cell line		DRB1																	
1	9001	SA	*0101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*1502	*0405	-	-	-	+	+	-	+	-	+	-	-	-	-	-	-	-
3	9011	E4181324	*1502		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*0301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*0407	*0803	-	-	+	-	-	-	+	-	+	+	-	-	-	-	-	+
7	9020	QBL	*0301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9007	DEM	*0401	*1602	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-
9	9026	YAR	*0402		-	+	-	+	-	-	-	+	-	+	-	-	-	+	-	-
10	9107	LKT3	*0405		-	-	-	+	+	-	+	-	+	-	-	-	-	-	-	-
11	9051	PITOUT	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9067	BTB	*0801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*0802		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*0901		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*1101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9008	WILJON	*1501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*0901	*1101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*1201		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*1302		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*1402		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*1302	*1401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*0803	*1414	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*1101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*0301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*0405	*1001	-	-	-	+	+	-	+	-	+	-	-	-	-	-	-	-
28	9320	BEL5GB	*0416	*0701	+	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-
29	9050	MOU	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*0302		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*0301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*1303		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*0404		-	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-
34	9104	DHIF	*1101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*0403		-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	+
36	9024	KT17	*0403	*0406	-	-	+	-	-	+	-	+	+	+	-	-	-	-	-	+
37	9065	HHKB	*1301		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*1402		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*0301	*0401	+	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-
40	9134	WHONP199	*0701	*0901	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*1302		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*0803		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*0901		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*1401		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*1501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*1104	*1201	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*0103		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CELL LINE VALIDATION SHEET																																		
DRB1*04 SSP subtyping kit																																		
					Tube																													
					17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32														
				Prod. No.	200736217	200736218	200736219	200736220	200736221	200736222	200736223	200736224	200736225	20036226	200736227	200736228	200736229	200736230	200736231	200736232														
	cell line		DRB1																															
1	9001	SA	*0101		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
2	9280	LK707	*1502	*0405	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-												
3	9011	E4181324	*1502		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
4	9275	GU373	*0301		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
5	9009	KAS011	*1601		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
6	9353	SM	*0407	*0803	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-													
7	9020	QBL	*0301		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
8	9007	DEM	*0401	*1602	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-													
9	9026	YAR	*0402		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
10	9107	LKT3	*0405		-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-													
11	9051	PITOUT	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
12	9052	DBB	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
13	9067	BTB	*0801		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
14	9071	OLGA	*0802		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
15	9075	DKB	*0901		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
16	9037	SWEIG007	*1101		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
17	9008	WILJON	*1501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
18	9257	32367	*0901	*1101	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
19	9038	BM16	*1201		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
20	9059	SLE005	*1302		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
21	9064	AMALA	*1402		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
22	9056	KOSE	*1302	*1401	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
23	9124	IHL	*0803	*1414	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
24	9035	JBUSH	*1101		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
25	9049	IBW9	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
26	9285	WT49	*0301		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
27	9191	CH1007	*0405	*1001	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-													
28	9320	BEL5GB	*0416	*0701	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-													
29	9050	MOU	*0701		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
30	9021	RSH	*0302		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
31	9019	DUCAF	*0301		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
32	9297	HAG	*1303		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-												
33	9098	MT14B	*0404		-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-													
34	9104	DHIF	*1101		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
35	9302	SSTO	*0403		-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-													
36	9024	KT17	*0403	*0406	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-													
37	9065	HHKB	*1301		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
38	9099	LZL	*1402		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
39	9315	CML	*0301	*0401	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-													
40	9134	WHONP199	*0701	*0901	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
41	9055	H0301	*1302		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
42	9066	TAB089	*0803		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
43	9076	T7526	*0901		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
44	9057	TEM	*1401		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
45	9239	SHJO	*0701		-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
46	9013	SCHU	*1501		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													
47	9045	TUBO	*1104	*1201	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-													
48	9303	TER-ND	*0103		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-													

CERTIFICATE OF ANALYSIS

Olerup SSP™ DRB1*04 SSP

Product number: 101.114-24/03 – licensed for PCR
101.114-24u/03u – not licensed for PCR
Lot number: Y08
Expiry date: 2009-July-01
Number of tests: 24 tests – Product No. 101.114-24
3 tests – Product No. 101.114-03
Number of tubes per test: 32

Tube specifications:

Tube No.	Production No.	Tube No.	Production No.	Tube No.	Production No.
1	2007-362-01	13	2007-362-13	25	2007-362-25
2	2007-362-02	14	2007-362-14	26	2007-362-26
3	2007-362-03	15	2007-362-15	27	2007-362-27
4	2007-362-04	16	2007-362-16	28	2007-362-28
5	2007-362-05	17	2007-362-17	29	2007-362-29
6	2007-362-06	18	2007-362-18	30	2007-362-30
7	2007-362-07	19	2007-362-19	31	2007-362-31
8	2007-362-08	20	2007-362-20	32	2007-362-32
9	2007-362-09	21	2007-362-21		
10	2007-362-10	22	2007-362-22		
11	2007-362-11	23	2007-362-23		
12	2007-362-12	24	2007-362-24		

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

No DNAs carrying the alleles to be amplified by primer solutions 11, 13, 15, 17 to 23, 25, 27 and 28 were available. The specificities of the primers in primer solutions 11, 13, 17, 18, 19, 20, 27 and 28 were tested by separately adding one additional 5'-primer, respectively, one additional 3'-primer. One of the 3'-primers in primer solutions 13, 14, 18, 19 and 20 was not possible to test. In primer solutions 15 and 25 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 21, 22 and 23 it was only possible to test the 5'-primers, the 3'-primers were not possible to test. Additional 3'-primers in primer solution 2, 9 and 12 were not possible to test.

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

Date of approval: 2007-September-06

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: Olerup SSP™ DRB1*04
Product number: 101.114-24/03, 101-114-24u/03u
Lot number: Y08

Intended use: DRB1*04 high 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 13485:2003, 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-September-06

Olle Olerup
Managing Director

DRB1*04

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101.114-24/03 – licensed for PCR

101.114-24u/03u – not licensed for PCR

Lot No.: **Y08**

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DRB1*04

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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.

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