

Olerup SSP® DRB1*04

Product number: 101.114-24u/03u – without *Taq* pol.
Lot number: 04G
Expiry date: 2011-July-01
Number of tests: 24 tests – Product No. 101.114-24u
 3 tests – Product No. 101.114-03u
Number of wells per test: 42
Storage - pre-aliquoted primers: dark at -20°C
- PCR Master Mix: -20°C

This Product Description is only valid for Lot No. 04G.

**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. 57E**) was made.

Two wells have been added to the DRB1*04 kit,
wells **41 to 42**.

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

Well	5'-primer	3'-primer	rationale
17	-	Removed	Primer pair moved to vial 42.
21	-	Added	New primer for the DRB1*0473 allele, exchanged positive control primer.
32	-	Added	Primer added for enhanced amplification of the DRB1*0433 allele.
38	New	New	New primer pair for the DRB1*0473 and *0475 alleles.
41	New	New	New primer pair for the DRB1*0476 allele.
42	Moved	Moved	Primer pair from well 17.

Change in revision R01 compared to R00:

1. The size of the positive control in primer mix 38 has been corrected to 515 bp in the specificity and interpretation tables.

Changes in revision R02 compared to R01:

1. The DRB1*040302 allele is weakly amplified by primer mix 10. This has been corrected in the Specificity and Interpretation Tables.

PRODUCT DESCRIPTION

DRB1*04 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*0401 to DRB1*0478 alleles.

PLATE LAYOUT

Each test consists of 42 PCR reactions in a 48 well cut PCR plate. Wells 43 to 48 are empty.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32
33	34	35	36	37	38	39	40
41	42	empty	empty	empty	empty	empty	empty

The 48 well cut PCR plate is marked with 'DRB1*04' in silver/gray ink.

Well No. 1 is marked with the Lot No. '04G'.

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

Please note: When removing each 48 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

Four DRB1*01, several DRB1*03, five DRB1*08, most DRB1*09, ten DRB1*11, many DRB1*13, several 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 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*0478**, recognized by the HLA Nomenclature Committee in May 2009¹ 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 to *040103 alleles, the DRB1*040301 to DRB1*040304 alleles, the DRB1*040501 and DRB1*040503 to DRB1*040506 alleles, the DRB1*040601 and DRB1*040602 alleles and the DRB1*040701 to DRB1*040703 alleles.

¹DRB1 alleles listed on the IMGT/HLA web page 2009-May-09, release 2.25.2, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 78 DRB1*04 alleles will give rise to 79 different amplification patterns. These can be combined in 3 160 homozygous and heterozygous combinations. 497 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 11 to 14, 19 to 21, 23, 25, 27, 29 and 31 were not considered in these calculations.)

++-+---++	-+---+---	----+---+	-----+	-----	--	0402,0438 = 0437,0438
+++++--+	++-----	-----	-++-+--+	-----	--	0406,0467 = 0449,0467
+++++--+	++-----	-----	-++-+--+	-----	--	0403,0467 = 0460,0467
+++++--+	++-----	-----	-++-+--+	-----	--	0416,0459 = 0435,0452
+++++--+	++-----	-----	-++-+--+	-----	--	0416,0427 = 0452,0463
+++++--+	++-----	-----	-++-+--+	-----	--	0401,0452 = 0403,0416
						= 0416,0452
++-+---++	++-----	-+-+---+	-+-----	-----	--	0427,0435 = 0459,0463
++-+---++	++-----	-+-+---+	-+-----	-----	--	0401,0459 = 0403,0435
						= 0435,0459
++-+---++	++-----	---+---+	-+-----	-----	--	0401,0427 = 0403,0463
						= 0427,0463
+++++--+	++-----	-----	-+-----	-----	--	0405,0413 =
						040502,0413
+++++--+	++-----	-----	-+-+---+	-----	--	0405,0467 =
						040502,0467
++-+---+	++-+---+	-----	-+-----	-----	--	0405,0416 =
						040502,0416
++-+---+	++-+---+	-----	-+-----	-----	--	0405,0438 =
						040502,0438 =
						0429,0438 = 0438,0448
++-+---+	++-----	-+-----	++-----	-----	--	0426,0428 = 0435,0445
++-+---+	++-----	-+-----	-+-----	-----	--	0401,0428 = 0405,0435
						= 040502,0435 =
						0428,0435
++-+---+	++-----	---+---+	-+-----	-----	--	0405,0463 =
						040502,0463
++-+---+	++-----	-----	-+-----	-----	--	0405,0433 =
						040502,0433
++-+---+	++-----	-----	++-----	-----	--	0401,0445 = 0405,0426
						= 040502,0426 =
						0426,0445
++-+---+	++-----	-----	-+-----	-----	--	0405,0472 =
						040502,0472
++-+---+	++-----	-----	-+-----	-----	+-	0405,0476 =
						040502,0476
++-+---+	++-----	-----	-+-----	-----	--	0401,0405 =
						0401,040502
++-+---+	++-----	-----	-+-----	-----	--	0405,0409 =
						040502,0409
++-+---+	++-----	-----	-+-----	-----	--	0405,0462 =
						040502,0462
++-+---+	++-+---+	-----	-+-----	-----	--	0416,0468 = 0421,0423
++-+---+	++-+---+	-----	-+-----	-----	--	0421,0456 = 0438,0468
++-+---+	++-+---+	-----	-+-----	-----	--	0421,0442 = 0435,0468
++-+---+	++-+---+	-----	-+-----	-----	--	0421,0440 = 0463,0468
++-+---+	++-+---+	-----	-+-----	-----	--	0421,0444 = 0433,0468
++-+---+	++-+---+	-----	-+-----	-----	--	0401,0468 = 0404,0421
++-+---+	++-+---+	-----	-+-----	-----	--	0419,0435 = 0421,0443
++-+---+	++-+---+	-----	-+-----	-----	--	0401,0419 = 0408,0421
++-+---+	++-+---+	-----	-+-----	-----	--	0416,0456 = 0423,0438
++-+---+	++-+---+	-----	-+-----	-----	--	0416,0442 = 0423,0435

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+++---++	++-+-----	---+---+	-+-----+	-----	--	0416,0440 = 0423,0463
+++---++	++-+-----	---+---+	-+-----+	-----	--	0416,0444 = 0423,0433
+++---++	++-+-----	---+---+	-+-----+	-----	--	0401,0423 = 0404,0416 = 0416,0423
+++---++	++-+---+	---+---+	-+-----+	-----	--	0418,0438 = 0438,0458
+++---++	++-+---+	---+---+	-+-----+	-----	--	0435,0456 = 0438,0442
+++---++	++-+---+	---+---+	-+-----+	-----	--	0438,0440 = 0456,0463
+++---++	++-+---+	---+---+	-+-----+	-----	--	0433,0456 = 0438,0444
+++---++	++-+---+	---+---+	-+-----+	-----	--	0401,0456 = 0404,0438
+++---++	++-+---+	---+---+	-+-----+	-----	--	0438,0453 = 0438,0456
+++---++	++-+---+	---+---+	-+-----+	-----	--	0435,0440 = 0442,0463
+++---++	++-+---+	---+---+	-+-----+	-----	--	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
+++---++	++-+---+	---+---+	-+-----+	-----	--	0401,0422 = 0422,0472
+++---++	++-+---+	---+---+	-+-----+	-----	--	0401,0443 = 0408,0435 = 0435,0443
+++---++	++-+---+	---+---+	-+-----+	-----	--	0401,0474 = 0433,0474
+++---++	++-+---+	---+---+	-+-----+	-----	--	0401,0416 = 0416,0416
+++---++	++-+---+	---+---+	-+-----+	-----	--	0401,0435 = 0435,0435
+++---++	++-+---+	---+---+	-+-----+	-----	--	0401,0463 = 0463,0463
+++---++	++-+---+	---+---+	-+-----+	-----	--	0401,0433 = 0433,0433
+++---++	++-+---+	---+---+	-+-----+	-----	--	0401,0464 = 0426,0464
+++---++	++-+---+	---+---+	-+-----+	-----	--	0401,0426 = 0426,0426
+++---++	++-+---+	---+---+	-+-----+	-----	--	0401,0472 = 0472,0472
+++---++	++-+---+	---+---+	-+-----+	-----	+	0401,0476 = 0476,0476
+++---++	++-+---+	---+---+	-+-----+	-----	--	0402,0429 = 0437,0448
+++---++	++-+---+	---+---+	-+-----+	-----	--	0402,0405 = 0402,040502 = 0402,0448
+++---++	++-+---+	---+---+	-+-----+	-----	--	0405,0437 = 040502,0437 = 0429,0437
+++---++	++-+---+	---+---+	-+-----+	-----	--	0405,0414 = 040502,0414 = 0414,0448
+++---++	++-+---+	---+---+	-+-----+	-----	--	0402,0458 = 0418,0437
+++---++	++-+---+	---+---+	-+-----+	-----	--	0402,0456 = 0437,0453 = 0437,0456
+++---++	++-+---+	---+---+	-+-----+	-----	--	0402,0473 = 0437,0473
+++---++	++-+---+	---+---+	-+-----+	-----	--	0405,0478 = 0406,0424 = 0424,0478
+++---++	++-+---+	---+---+	-+-----+	-----	--	0406,0410 = 0410,0449
+++---++	++-+---+	---+---+	-+-----+	-----	--	0405,0452 = 040502,0452
+++---++	++-+---+	---+---+	-+-----+	-----	--	0424,0459 = 0428,0439
+++---++	++-+---+	---+---+	-+-----+	-----	--	0403,0428 = 0405,0459 = 040502,0459 = 0428,0459
+++---++	++-+---+	---+---+	-+-----+	-----	--	0403,0424 = 0405,0439 = 040502,0439 = 0424,0439
+++---++	++-+---+	---+---+	-+-----+	-----	--	0405,0427 = 040502,0427

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--++++++	++-----	-----+	-+-----	-----	--	0405,0450 =	
						040502,0450	
--++++++	++-----	-----	-++-----	-----	--	0405,0460 =	
						040502,0460	
--++++++	++-----	-----	-+-----	--+-----	--	0405,0451 =	
						040502,0451	
--++++++	++-----	-----	-+-----	---+-----	--	0405,0471 =	
						040502,0471	
--++++++	++-----	-----	-+-----	-----	--	0403,0405 =	
						0403,040502	
--++++++	++-----	-----	-+-----	-----	+	0405,0441 =	
						040502,0441 =	
						0441,0477	
--++++++	+-----	-----	-+++-----	-----	--	0405,0411 =	
						040502,0411	
--+++++-	++-----	-----	-+-+-----	-----	--	0405,0407 =	
						040502,0407	
--+++++-	+-----	--+-----	-+-+-----	-----+	--	0405,0469 =	
						040502,0469 =	
						0424,0469	
--+++++-	+-----	-----	-+-+-----	-----	--	0405,0417 =	
						040502,0417	
--++++-+	++-----	-----	-++-----	-----	--	0403,0410 =	0410,0460
--++++-+	++-----	-+-----	-+-----	-----	--	0403,0443 =	0408,0459
						= 0443,0459	
--++-----	++-+-----	-+-----	-+-----	-----	--	0423,0459 =	0442,0452
--++-----	++-+-----	---+-----	-+-----	-----	--	0423,0427 =	0440,0452
--++-----	++-+-----	-----+	-+-----	-----	--	0423,0450 =	0432,0452
--++-----	++-+-----	-----	-+-----	--+-----	--	0423,0451 =	0452,0470
--++-----	++-+-----	-----	-+-----	-----	--	0403,0423 =	0404,0452
						= 0423,0452	
--++-----	++-----	-+-+-----	-+-----	-----	--	0427,0442 =	0440,0459
--++-----	++-----	-+-+-----	-+-----	-----	--	0432,0459 =	0442,0450
--++-----	++-----	-+-----	-+-----	--+-----	--	0442,0451 =	0459,0470
--++-----	++-----	-+-----	-+-----	-----	--	0403,0442 =	0404,0459
						= 0442,0459	
--++-----	++-----	---+-----	-+-----	-----	--	0427,0432 =	0440,0450
--++-----	++-----	---+-----	-+-----	--+-----	--	0427,0470 =	0440,0451
--++-----	++-----	---+-----	-+-----	-----	--	0403,0440 =	0404,0427
						= 0427,0440	
--++-----	++-----	-----+	-+-----	--+-----	--	0432,0451 =	0450,0470
--++-----	++-----	-----+	-+-----	-----	--	0403,0432 =	0404,0450
						= 0432,0450	
--++-----	++-----	-----	-+-----	--+-----	--	0403,0470 =	0404,0451
						= 0451,0470	
--++++++	++-----	-----	-++++-----	-----	--	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
--++-+++	++-----	---+-----	-+-+-----	-----+	--	0406,0469 =	0469,0478
--++-+++	++++++-	---+-----	-+-+-----	-----	--	0407,0478 =	0420,0439
--++-+++	++-----	-----	-+-+-----	-----	--	0407,0449 =	0420,0460
--++-+++	++-----	-----	-+-+-----	-----	--	0403,0420 =	0406,0407
--++-++-	++-++-	-----	-+-----	-----	--	0406,0418 =	0418,0446
--++-++-	++-+-----	+-----	-+-----	-----	--	0406,0425 =	0425,0446

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--+---++	++---+---	-----+---	-+-----+	-----	--	0406,0458 = 0446,0458
--+---++	++---+---	-----	-+-----+	-----	--	0406,0455 = 0446,0455
--+---++	++---+---	-----	-+-----+	-----	--	0406,0446 = 0446,0446
--+---++	++-----+	--+-----	-+-----+	-----	--	0439,0449 = 0460,0478
--+---++	++-----+	--+-----	-+-----+	-----	--	0403,0478 = 0406,0439 = 0439,0478
--+---++	++-----+	-----	-+-----+	-----	--	0403,0449 = 0406,0460 = 0449,0460
--+---++	++-----	--+-----	-+-----	-----	--	0406,0478 = 0478,0478
--+---++	++-----	-----	-+-----	-----	--	0406,0449 = 0449,0449
--+---++	++-----+	--+-----	-+---++	-----+	--	0403,0469 = 0439,0469
--+---++	++---+---	-----	-+-----+	-----	--	0403,0452 = 0452,0452
--+---++	++-----+	-+-----	-+-----+	-----	--	0403,0459 = 0459,0459
--+---++	++-----+	--+-----	-+-----+	-----	--	0403,0439 = 0439,0439
--+---++	++-----+	--+-----	-+-----+	-----	--	0403,0427 = 0427,0427
--+---++	++-----+	-----+	-+-----+	-----	--	0403,0450 = 0450,0450
--+---++	++-----+	-----	-+-----+	-----	--	0403,0460 = 0460,0460
--+---++	++-----+	-----	-+-----+	-----	--	0403,0451 = 0451,0451
--+---++	++-----+	-----	-+-----+	-----	--	0403,0471 = 0471,0471
---++++	+++-----	-----	-+-----+	-----+	--	0405,0422 = 040502,0422
---++++	+++-----	-----	-+-----+	-----	--	0405,0423 = 040502,0423
---++++	+++---+---	+-----	-+-----+	-----	--	0418,0457 = 0425,0448
---++++	+++---+---	-----+	-+-----+	-----	--	0418,0429 = 0448,0458
---++++	+++---+---	-----	-+-----+	-----	--	0405,0418 = 040502,0418 = 0418,0448 = 0448,0455
---++++	+++---+---	+---+---	-+-----+	-----	--	0425,0429 = 0457,0458
---++++	+++---+---	+-----	-+-----+	-----	--	0405,0425 = 040502,0425 = 0425,0457 = 0455,0457
---++++	+++---+---	-----+	-+-----+	-----	--	0405,0458 = 040502,0458 = 0429,0455 = 0429,0458
---++++	+++---+---	-----	-+-----+	-----	--	0405,0455 = 040502,0455
---++++	+++---+---	+-----	-+-----+	-----	--	0436,0448 = 0453,0457
---++++	+++---+---	-+-----	-+-----+	-----	--	0428,0453 = 0442,0448
---++++	+++---+---	-----+	-+-----+	-----	--	0405,0456 = 040502,0456 = 0429,0453 = 0429,0456 = 0448,0456
---++++	+++---+---	-----	-+-----+	-----	--	0404,0448 = 0405,0453 = 040502,0453 = 0448,0453
---++++	+++-----	++-----	-+-----+	-----	--	0428,0436 = 0442,0457
---++++	+++-----	+-----	-+-----+	-----	--	0404,0457 = 0405,0436 = 040502,0436 = 0436,0457
---++++	+++-----	-+-----	-+-----+	-----	--	0404,0428 = 0405,0442 = 040502,0442 = 0428,0442
---++++	+++-----	-----+	-+-----+	-----	--	0405,0440 = 040502,0440
---++++	+++-----	-----+	-+-----+	-----	--	0405,0432 = 040502,0432
---++++	+++-----	-----+	-+-----+	-----	--	0405,0444 = 040502,0444

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---++---++ ++----- ----- -+-----+ ---+----- -- 0405,0470 =
040502,0470
---++---++ ++----- ----- -+-----+ ----- -- 0404,0405 =
0404,040502
---++---++ +-+----- +----- -+-----+ ----- -- 0405,0415 =
040502,0415 =
0415,0457
---++---++ +---++--- ----- -+-----+ ----- -- 0405,0412 =
040502,0412 =
0412,0448
---++---++ +-----+--- -----+----- -+-----+ -----+--- -- 0405,0473 =
040502,0473 =
0429,0473 = 0448,0473
---++---++ +----- ----- ----- -+-----+ ----- -- 0405,0410 =
040502,0410
---++---++ +-+---+--- +----- -+-----+ ----- -- 0405,0454 =
040502,0454 =
0431,0457 = 0454,0457
---++---++ +-+---+--- ----- -+-----+ ----- -- 0405,0431 =
040502,0431
---++---++ ++----- ++----- -+-----+ ----- -- 0428,0447 = 0443,0457
---++---++ ++----- +----- -+-----+ ----- -- 0405,0447 =
040502,0447 =
0408,0457 = 0447,0457
---++---++ ++----- -+----- -+-----+ ----- -- 0405,0443 =
040502,0443 =
0408,0428 = 0428,0443
---++---++ ++----- -----+--- -+---+---+ ----- -- 0405,0474 =
040502,0474
---++---++ ++----- ----- -+-----+ -----+--- -- 0405,0475 =
040502,0475
---++---++ ++----- ----- -+-----+ ----- -- 0405,0408 =
040502,0408
---++---++ +-+----- ----- -+-----+ ----- -- 0405,0461 =
040502,0461
---++---++ +-----+--- ----- -+-----+ ----- -- 0405,0448 =
040502,0448 =
0448,0448
---++---++ +----- +----- -+-----+ ----- -- 0405,0457 =
040502,0457 =
0457,0457
---++---++ +----- -+----- -+-----+ ----- -- 0405,0428 =
040502,0428 =
0428,0428
---++---++ +----- --+----- -+-----+ ----- -- 0405,0424 =
040502,0424 =
0424,0424
---++---++ +----- -----+--- -+-----+ ----- -- 0405,0429 =
040502,0429 =
0429,0429
---++---++ +----- -----+ ++---+---+ ----- -- 0405,0464 = 0445,0464
---++---++ +----- ----- ++-----+ ----- -- 0405,0445 =
040502,0445 =
0445,0445
---++---++ +----- ----- -+-----+ -----+ --- 0405,0430 =
040502,0430 =
0430,0430
---++---++ +----- ----- -+-----+ ----- --+ 0405,0477 =
040502,0477 =
0477,0477

```


SPECIFICITY TABLE

DRB1*04 SSP subtyping

Specificities and sizes of the PCR products of the 42 primer mixes used for DRB1*04 SSP subtyping

Primer Mix	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	040101-040103, 0409, 0413, 0416, 0421, 0426, 0433, 0435, 0438, 0462, 0463, 0467, 0472, 0476	
2	215 bp	430 bp	0402, 0414, 0437	1457
3	225 bp	430 bp	040301-040304, 040601-040703, 0411, 0417, 0420, 0427, 0439, 0441, 0446, 0449-0452, 0459, 0460, 0465, 0469, 0471, 0478	1410
4	225 bp	515 bp	040101-0402, 0404-040506, 0408-0410, 0413-0416, 0419, 0421, 0423, 0424, 0426, 0428-0430, 0432, 0433, 0435-0438, 0440, 0442, 0443, 0444 ^{weakly} , 0445, 0447, 0448, 0453, 0456, 0457, 0461-0463, 0467, 0468, 0470, 0472, 0473, 0475-0477	1122, 1457
5	170 bp	430 bp	040501-040506, 0409-0412, 0417, 0424, 0428-0430, 0445, 0448, 0457, 0467, 0477	
6⁵	115 bp	430 bp	040601-040602, 0419-0421, 0446, 0449, 0465, 0468, 0478	
7	260 bp	430 bp	040101-040103, 040501-040506, 040701-0409, 0414, 0416, 0417, 0419-0421, 0424, 0426, 0428-0431, 0433, 0435, 0438, 0443, 0445, 0447, 0448, 0454, 0457, 0461-0463, 0469, 0472, 0474-0477	1122
8	260 bp	430 bp	0402-0404, 040601-	1410, 1457

			040602, 0410-0413, 0415, 0418, 0422, 0423, 0425, 0427, 0432, 0436, 0437, 0439-0442, 0444, 0446, 0449-0453, 0455, 0456, 0458-0460, 0465, 0467, 0468, 0470, 0471, 0473, 0478	
9⁶	215 bp	430 bp	040301-0408, 0410, 0411, 0412 ^{weakly} , 0415, 0417, 0418 ^{weakly} , 0419, 0420, 0423, 0424, 0425 ^{weakly} , 0427-0430, 0431 ^{weakly} , 0432, 0436, 0439- 0446, 0447 ^{weakly} , 0448- 0452, 0453-0455 ^{weakly} , 0456, 0457, 0458 ^{weakly} , 0459-0461, 0468-0471, 0474, 0477, 0478	1122, 1410
10	170 bp	515 bp	040101-040301, 040302 ^{weakly} , 0404, 040601-0408, 0413, 0414, 0416, 0418-0423, 0425, 0426 ^{weakly} , 0427, 0431-0433, 0435-0444, 0446, 0447, 0449-0456, 0458-0460, 0463, 0465, 0468, 0470-0472, 0474- 0476, 0478	
11⁹	175, 220 bp	430 bp	0415, 0422, 0461	1122
12¹⁰	180, 265 bp	515 bp	0416, 0423, 0452	
13¹¹	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, 0473 ^{weakly}	1457
15⁶	215 bp	430 bp	0434	
16^{6,7,8}	150 bp	515 bp	040301-040304, 040701-040703, 0411, 0417, 0427, 0439, 0450 ^{weakly} , 0451, 0452, 0459, 0460, 0469, 0471	0829 ^{weakly} , 1153 - 115402 ^{weakly} , 1442 ^{weakly} , 1458 ^{weakly}
17	190 bp	430 bp	0415, 0425, 0436, 0447, 0454, 0457	1122
18⁶	145 bp	430 bp	0428, 0435, 0442, 0443, 0459	1122
19^{6,13}	140, 210 bp	515 bp	0424, 0439, 0469, 0478	1410

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20 ^{5,14}	100, 175, 255 bp	430 bp	0427, 0440, 0463	
21 ¹⁵	155, 200 bp	430 bp	0429, 0437, 0438, 0456, 0458, 0473	
22	230 bp	430 bp	0432, 0450	
23 ^{5,16}	120, 235 bp	430 bp	0433, 0444, 0474	
24 ⁵	85 bp	430 bp	040101-040103, 0409, 0416, 0421, 0426, 0433-0435, 0438, 0462-0464, 0466, 0472, 0476	0110, 0116, 030201-030202, 030501-030502, 0309, 0314, 0317, 0327, 0329, 0335, 0338 ^{weakly} , 0340, 0341, 1153 ^{weakly} , 1419, DRB3*01010201-0113, DRB3*020201-0203, 0205-0210, 0211^{weakly}, 0212, 0213, 0215, 0216, 0217^{weakly}, 0218-0223, DRB3*0303
25 ^{5,17}	125, 230, 260 bp	430 bp	0426, 0445, 0464	
26	200 bp	515 bp	040101-040103, 040301-0411, 0413, 0416, 0417, 0419-0424, 0426-0433, 0435, 0439-0446, 0448-0452, 0455, 0457, 0459-0463, 0465, 0467-0472, 0474-0478	1410
27 ^{5,6,18}	120, 265 bp	515 bp	0410-0412, 0449, 0460, 0467	0312, 0806, 0810, 0812, 0822, 1304, 1332, 1348, 1358, 1375, 1381, 1465, 1478, 1512
28 ⁵	90 bp	430 bp	0411, 0417	0829
29 ^{5,19}	75, 260 bp	430 bp	040701-040703, 0417, 0420, 0464, 0469, 0474	0117, 090102-090202, 0904, 0905, 0907, 0908, 140701-140702, 1414, 1436, 1442, 144401-144402,

				1451, 1468, DRB5*0112
30⁵	80 bp	430 bp	0409, 0467	130301-1304, 1332, 1333, 1338, 1348, 1365, 1366, 1375, 1381, 1388
31^{5,7,20}	85, 260 bp	430 bp	0414, 0464	0103, 111101- 111102, 111401- 111402, 1120, 1168, 130201- 130302, 1323, 1329, 1331, 1333, 1334, 1336-1339, 1341, 1345, 1363, 1365-1367, 1373, 1374, 1385, 1388
32⁵	115 bp	430 bp	040101-040501, 040503-040506, 040701-0418, 0422- 0433, 0435-0445, 0447, 0448, 0450-0463, 0467, 0469-0477	1122
33	215 bp	430 bp	0465	
34	260 bp	515 bp	0466	
35	245 bp	430 bp	0451, 0470	
36	240 bp	515 bp	0471	
37	235 bp	430 bp	0422, 0472	
38⁷	215 bp	515 bp	0473, 0475	
39	175 bp	430 bp	0462, 0469	1410, 1457
40	150 bp	430 bp	0430	
41	160 bp	430 bp	0476	
42⁵	110 bp	430 bp	0441, 0477	

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 DRB*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 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective length of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

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

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low

amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

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

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB1*04 subtyping.

In addition, wells number 4, 10, 12, 16, 19, 26, 27, 34, 36 and 38 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

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

³For 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.

⁴The DRB1*1122, DRB1*1410 and DRB1*1457 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, four DRB1*01, several DRB1*03, five DRB1*08, most DRB1*09, ten DRB1*11, many DRB1*13, several 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 19, 24, 26 to 32 and 39, but the purpose of these primer mixes is to increase the resolution in DRB1*04 homozygotes.

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

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

⁷Primer mixes 14, 16, 31 and 38 have a tendency of giving rise to primer dimer formation, most pronounced in primer mix 38.

⁸Primer mix 16 may yield somewhat less specific PCR fragment than the other DRB1*04 primer mixes.

⁹Primer 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.

¹⁰Primer 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.

¹¹Primer 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.

¹²Primer mix 14: Specific PCR fragment of 200 bp in the DRB1*0402, 0412, 0414, 0418, 0438, 0453, 0456, 0473^{weakly} and the DRB1*1457 alleles. Specific PCR fragment of 220 bp in the DRB1*0448 allele.

¹³Primer mix 19: Specific PCR fragment of 140 bp in the DRB1*0439 allele. Specific PCR fragment of 210 bp in the DRB1*0424, 0469, 0478 and the DRB1*1410 alleles.

¹⁴Primer 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.

¹⁵Primer 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, 0458 and 0473 alleles.

¹⁶Primer mix 23: Specific PCR fragment of 120 bp in the DRB1*0433 allele. Specific PCR fragment of 235 bp in the DRB1*0444 and 0474 alleles.

¹⁷Primer 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.

¹⁸Primer mix 27: Specific PCR fragment of 120 bp in the DRB1*0410 to 0412, 0460, 0467 and the DRB1*0312, 0806, 0810, 0812, 0822, 1304, 1332, 1348, 1358, 1375, 1381, 1465, 1478 and 1512 alleles. Specific PCR fragment of 265 bp in the DRB1*0449 allele.

¹⁹Primer mix 29: Specific PCR fragment of 75 bp in the DRB1*040701-040703, 0417, 0420, 0469, 0474 and the DRB1*0117, 090102-090202, 0904, 0905, 0907, 0908, 140701-140702, 1414, 1436, 1442, 144401-144402, 1451, 1468 and the DRB5*0112 alleles. Specific PCR fragment of 260 bp in the DRB1*0464 allele.

²⁰Primer mix 31: Specific PCR fragment of 85 bp in the DRB1*0414 and the DRB1*0103, 111101-111102, 111401-111402, 1120, 1168, 130201-130302, 1323, 1329, 1331, 1333, 1334, 1336-1339, 1341, 1345, 1363, 1365-1367, 1373, 1374, 1385 and 1388 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 *0478 alleles**

	Well ⁴																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Length of spec. PCR product	215	215	225	225	170	115	260	260	215	170	175	180	170	200	215	150	190	145	140	100
											220	265	225	220					210	175
																				255
Length of int. pos. control¹	515	430	430	515	430	430	430	430	430	515	430	515	430	430	430	515	430	430	515	430
5'-primer(s)²	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	37	13	13	13	13
	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 CC ³	5'-g TA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³	5'-A CA ³
3'-primer(s)³	71	71	74	74	57	37	86	86	71	57	58	59	56	67	71	74	60	47	46	33
	5'-CTT ³	5'-CTC ³	5'-C CT ³	5'-C Cg ³	5'-g CT ³	5'-C gg ³	5'-C AC ³	5'-C CA ³	5'-TCT ³	5'-ATC ³	5'-C CT ³	5'-CTg ³	5'-AgC ³	5'-gAT ³	5'-CTT ³	5'-C CT ³	5'-gTT ³	5'-g gA ³	5'-TA g ³	5'-gTT ³
									71	57	73	88	74	67			67		70	58
									5'-CCT ³	5'-ATC ³	5'-g gC ³	5'-AA T ³	5'-CA g ³	5'-gAT ³			5'-gAA ³		5'-T CC ³	5'-C gC ³
														67						85
														5'-ggA ³						5'-C Ag ³
														73						
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
*040101-040103	1			4			7			10										
*0402		2		4				8		10				14						
*040301, 040403-040304			3					8	9	10						16				
*040302			3					8	9	w						16				
*0404				4				8	9	10										
*040501, 040503-040506				4	5		7		9											
*040502				4	5		7		9											
*040601-040602			3			6		8	9	10										
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

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Length of spec.	215	215	225	225	170	115	260	260	215	170	175	180	170	200	215	150	190	145	140	100
PCR product											220	265	225	220					210	175
																				255
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
DRB1 allele																				
*040701-040703			3				7		9	10					16					
*0408				4			7		9	10										
*0409	1			4	5		7													
*0410				4	5			8	9											
*0411			3		5			8	9						16					
*0412					5			8	w				13	14						
*0413	1			4				8		10										
*0414		2		4			7			10				14						
*0415				4				8	9		11						17			
*0416	1			4			7			10		12								
*0417			3		5		7		9						16					
*0418								8	w	10			13	14						
*0419				4		6	7		9	10										
*0420			3			6	7		9	10										
*0421	1			4		6	7			10										
*0422								8		10	11									
*0423				4				8	9	10		12								
*0424				4	5		7		9										19	
*0425								8	w	10			13				17			
*0426	1			4			7			w										
*0427			3					8	9	10					16					20
*0428				4	5		7		9								18			
*0429				4	5		7		9											
*0430				4	5		7		9											
*0431							7		w	10			13							
*0432				4				8	9	10										
*0433	1			4			7			10										
*0434															15					
*0435	1			4			7			10							18			
*0436				4				8	9	10							17			
*0437		2		4				8		10										
*0438	1			4			7			10			14							
*0439			3					8	9	10					16			19		
*0440				4				8	9	10										20
*0441			3					8	9	10										
*0442				4				8	9	10							18			
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

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155	230	120	85	125	200	120	90	75	80	85	115	215	260	245	240	235	215	175	150	160	110	Length of spec. PCR product
200		235		230		265		260		260												
				260																		
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Well No. DRB1 allele
					26			29			32											*040701-040703
					26						32											*0408
			24		26				30		32											*0409
					26	27					32											*0410
					26	27	28				32											*0411
						27					32											*0412
					26						32											*0413
										31	32											*0414
			24								32											*0415
					26						32											*0416
					26		28	29			32											*0417
											32											*0418
					26						32											*0419
					26			29														*0420
			24		26																	*0421
					26						32					37						*0422
					26						32											*0423
					26						32											*0424
											32											*0425
			24	25	26						32											*0426
					26						32											*0427
					26						32											*0428
21					26						32											*0429
					26						32								40			*0430
					26						32											*0431
	22				26						32											*0432
		23	24		26						32											*0433
			24																			*0434
			24		26						32											*0435
											32											*0436
21											32											*0437
21			24								32											*0438
					26						32											*0439
					26						32											*0440
					26						32									42		*0441
					26						32											*0442
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Well No.

Length of spec.	215	215	225	225	170	115	260	260	215	170	175	180	170	200	215	150	190	145	140	100
PCR product											220	265	225	220					210	175
																				255
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
*0443				4			7		9	10								18		
*0444				w				8	9	10										
*0445				4	5		7		9											
*0446			3			6		8	9	10			13							
*0447				4			7		w	10							17			
*0448				4	5		7		9				14							
*0449			3			6		8	9	10										
*0450			3					8	9	10						w				
*0451			3					8	9	10						16				
*0452			3					8	9	10		12				16				
*0453				4				8	w	10				14						
*0454							7		w	10			13				17			
*0455								8	w	10			13							
*0456				4				8	9	10				14						
*0457				4	5		7		9								17			
*0458								8	w	10			13							
*0459			3					8	9	10						16		18		
*0460			3					8	9	10						16				
*0461				4			7		9		11									
*0462	1			4			7													
*0463	1			4			7			10										20
*0464																				
*0465			3			6		8		10										
*0466																				
*0467	1			4	5			8												
*0468				4		6		8	9	10										
*0469			3				7		9							16			19	
*0470				4				8	9	10										
*0471			3					8	9	10						16				
*0472	1			4			7			10										
*0473				4				8					w							
*0474							7		9	10										
*0475				4			7			10										
*0476	1			4			7			10										
*0477				4	5		7		9											
*0478			3			6		8	9	10									19	
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

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155	230	120	85	125	200	120	90	75	80	85	115	215	260	245	240	235	215	175	150	160	110	Length of spec. PCR product
200		235		230		265		260		260												
				260																		
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Well No.
					26						32											*0443
		23			26						32											*0444
				25	26						32											*0445
					26																	*0446
											32											*0447
					26						32											*0448
					26	27																*0449
	22				26						32											*0450
					26						32			35								*0451
					26						32											*0452
											32											*0453
											32											*0454
					26						32											*0455
21											32											*0456
					26						32											*0457
21											32											*0458
					26						32											*0459
					26	27					32											*0460
					26						32											*0461
	24				26						32							39				*0462
			24		26						32											*0463
			24	25				29		31												*0464
					26							33										*0465
	24											34										*0466
					26	27			30		32											*0467
					26																	*0468
					26			29			32							39				*0469
					26						32			35								*0470
					26						32				36							*0471
			24		26						32					37						*0472
21											32						38					*0473
	23				26			29			32											*0474
					26						32						38					*0475
			24		26						32									41		*0476
					26						32										42	*0477
					26																	*0478
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Well No.

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Length of spec.	215	215	225	225	170	115	260	260	215	170	175	180	170	200	215	150	190	145	140	100
PCR product											220	265	225	220					210	175
																				255
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
*0103, 111101- 111102, 111401- 111402, 1120, 1168, 130201- 130203, 1323, 1329, 1331, 1334, 1336, 1337, 1339, 1341, 1345, 1363, 1367, 1373, 1374, 1385																				
*0110, 0116, 030201-030202, 030501-030502, 0309, 0314, 0317, 0327, 0329, 0335, 0340, 0341, 1419																				
*0117, 090102- 090202, 0904, 0905, 0907, 0908, 140701-140702, 1414, 1436, 144401-144402, 1451, 1468																				
*0312, 0806, 0810, 0812, 0822, 1358, 1465, 1478, 1512																				
*0338																				
*0829																w				
*1122				4			7		9		11						17	18		
*1153																w				
*115401-115402, 1458																w				
*130301-130302, 1333, 1338, 1365, 1366, 1388																				
*1304, 1332, 1348, 1375, 1381																				
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

Lot No.: **04G**

Lot-specific information

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155	230	120	85	125	200	120	90	75	80	85	115	215	260	245	240	235	215	175	150	160	110	Length of spec. PCR product
200		235		230		265		260		260												
				260																		
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Well No.
										31												*0103, 111101- 111102, 111401- 111402, 1120, 1168, 130201- 130203, 1323, 1329, 1331, 1334, 1336, 1337, 1339, 1341, 1345, 1363, 1367, 1373, 1374, 1385
			24																			*0110, 0116, 030201-030202, 030501-030502, 0309, 0314, 0317, 0327, 0329, 0335, 0340, 0341, 1419
								29														*0117, 090102- 090202, 0904, 0905, 0907, 0908, 140701-140702, 1414, 1436, 144401-144402, 1451, 1468
						27																*0312, 0806, 0810, 0812, 0822, 1358, 1465, 1478, 1512
			W																			*0338
							28															*0829
											32											*1122
			W																			*1153
																						*115401-115402, 1458
										30 31												*130301-130302, 1333, 1338, 1365, 1366, 1388
						27				30												*1304, 1332, 1348, 1375, 1381
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Well No.

Length of spec.	215	215	225	225	170	115	260	260	215	170	175	180	170	200	215	150	190	145	140	100
PCR product											220	265	225	220					210	175
																				255
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
*1410			3					8	9										19	
*1442																W				
*1457		2		4				8						14						
DRB1 allele																				
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
DRB3*01010201-0113, 020201-0203, 0205-0210, 0212, 0213, 0215, 0216, 0218-0223, 0303																				
DRB3*0211, 0217																				
DRB5*0112																				
Well No.	1	2	3	4	5	6	7	8	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 wells, or a band of 515 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the longer, 515 bp, internal positive control band in order to help in the correct orientation of the DRB1*04 subtyping.

In addition, wells number 4, 10, 12, 16, 19, 21, 26, 27, 34, 36 and 38 contain the primer pair giving rise to the longer, 515 bp, internal positive control band in order to allow kit identification.

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

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

155	230	120	85	125	200	120	90	75	80	85	115	215	260	245	240	235	215	175	150	160	110	Length of spec. PCR product
200		235		230		265		260		260												
				260																		
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Well No.
					26													39				*1410
								29														*1442
																		39				*1457
																						DRB1 allele
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Well No.
			24																			DRB3*01010201- 0113, 020201- 0203, 0205-0210, 0212, 0213, 0215, 0216, 0218-0223, 0303
			w																			DRB3*0211, 0217
								29														DRB5*0112
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Well No.

⁴Primer 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, 0473^{weakly} and the DRB1*1457 alleles. Specific PCR fragment of 220 bp in the DRB1*0448 allele.

Primer mix 19: Specific PCR fragment of 140 bp in the DRB1*0439 allele. Specific PCR fragment of 210 bp in the DRB1*0424, 0469, 0478 and the DRB1*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, 0458 and 0473 alleles.

Primer mix 23: Specific PCR fragment of 120 bp in the DRB1*0433 allele. Specific PCR fragment of 235 bp in the DRB1*0444 and 0474 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 to 0412, 0460, 0467 and the DRB1*0312, 0806, 0810, 0812, 0822, 1304, 1332, 1348, 1358, 1375, 1381, 1465, 1478 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, 0469, 0474 and the DRB1*0117, 090102-090202, 0904, 0905, 0907, 0908, 140701-140702, 1414, 1436, 1442, 144401-144402, 1451, 1468 and the DRB5*0112 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, 1168, 130201-130302, 1323, 1329, 1331, 1333, 1334, 1336-1339, 1341, 1345, 1363, 1365-1367, 1373, 1374, 1385 and 1388 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															
					Well										
						33	34	35	36	37	38	39	40	41	42
					Prod. No.	200846133	200846134	200846135	200846136	200846137	200961338	200846139	200846140	200961341	200961342
	IHWC cell line		DRB1												
1	9001	SA	*0101			-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*1502	*0405		-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*1502			-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*0301			-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*1601			-	-	-	-	-	-	-	-	-	-
6	9353	SM	*0407	*0803		-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*0301			-	-	-	-	-	-	-	-	-	-
8	9025	DEU	*0401			-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*0402			-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*0405			-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*0701			-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*0701			-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*0101			-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*0802			-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*0901			-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*1101			-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*0301	*1301		-	-	-	-	-	-	-	-	-	-
18	9257	32367	*0901	*1101		-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*1201			-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*1302			-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*1402			-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*1302	*1401		-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*0803	*1414		-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*1101			-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*0701			-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*0301			-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*0405	*1001		-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*0416	*0701		-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*0701			-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*0302			-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*0301			-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*1303			-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*0404			-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*1101			-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*0403			-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*0403	*0406		-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*1301			-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*1402			-	-	-	-	-	-	-	-	-	-
39	9315	CML	*0301	*0401		-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*0701	*0901		-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*1302			-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*0803			-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*0901			-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*1401			-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*0701			-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*1501			-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*1104	*1201		-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*0103			-	-	-	-	-	-	-	-	-	-

CERTIFICATE OF ANALYSIS

Olerup SSP® DRB1*04 SSP

Product number: 101.114-24u/03u – without *Taq* pol.
Lot number: 04G
Expiry date: 2011-July-01
Number of tests: 24 tests – Product No. 101.114-24u
 3 tests – Product No. 101.114-03u
Number of wells per test: 42

Well specifications:

Well No.	Production No.	Well No.	Production No.	Well No.	Production No.
1	2007-362-01	17	2009-613-17	33	2008-461-33
2	2008-461-02	18	2007-362-18	34	2008-461-34
3	2007-362-03	19	2008-461-19	35	2008-461-35
4	2007-362-04	20	2009-613-20	36	2008-461-36
5	2007-362-05	21	2009-613-21	37	2008-461-37
6	2007-362-06	22	2009-613-22	38	2009-613-38
7	2007-362-07	23	2008-461-23	39	2008-461-39
8	2007-362-08	24	2008-461-24	40	2008-461-40
10	2007-362-10	26	2007-362-26	41	2009-613-41
11	2007-362-11	27	2007-362-27	42	2009-613-42
12	2008-461-12	28	2007-362-28		
13	2007-362-13	29	2007-362-29		
14	2009-613-14	30	2007-362-30		
15	2007-362-15	31	2007-362-31		
16	2008-461-16	32	2009-613-32		

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

No DNAs carrying the alleles to be amplified by primer solutions 11, 13, 15, 17 to 20, 22, 23, 25, 27, 28 and 33 to 42 were available. The specificities of the primers in primer solutions 11, 13, 17 to 20, 23, 27, 28, 35, 37 to 39 and 42 were tested by separately adding one additional 5'-primer, respectively, one additional 3'-primer.

In primer solutions 15, 25, 34 and 36 it was only possible to test the 3'-primers, the 5'-primers were not possible to test.

In primer solutions 21, 22, 33, 40 and 41 it was only possible to test the 5'-primers, the 3'-primers were not possible to test.

One of the 3'-primers in primer solutions 12, 14, 17, 19, 20, 23 and 32 was not possible to test, and in primer solution 27, 29 and 31 one or two 5'-primers was not possible to test.

One additional 3'-primer in primer solution 21 was tested by separately adding one additional 5'-primer.

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

Date of approval: 2011-March-17

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*04

Product number: 101.114-24u/03u

Lot number: 04G

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
2011-March-17

Olle Olerup
Managing Director

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