

Olerup SSP[®] DRB1*09

| | |
|----------------------------------|---|
| Product number: | 101.120-06u – without <i>Taq</i> polymerase |
| Lot number: | 18F |
| Expiry date: | 2010-October-01 |
| Number of tests: | 6 |
| Number of wells per test: | 8 |
| Storage - pre-aliquoted primers: | dark at -20°C |
| - PCR Master Mix: | -20°C |
| - Adhesive PCR seals | RT |
| - Product Insert | RT |

This Product Description is only valid for Lot No. 18F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP[®]* DRB1*09 LOT

The DRB1*09 specificity and interpretation tables are unchanged compared to the previous *Olerup SSP[®]* DRB1*09 lot (**Lot No. Y45**).

One well has been added to the DRB1*09 kit,
well **8**.

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

| Well | 5'-primer | 3'-primer | rationale |
|------|-----------|-----------|---|
| 8 | New | New | New primer pair for the DRB1*0907 allele. |

PRODUCT DESCRIPTION

DRB1*09 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB1*0901 to DRB1*0907 alleles.

PLATE LAYOUT

Each test consists of 7 PCR reactions in an 8 well PCR plate.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|

The 8 well PCR plate is marked with 'DR9' in silver/gray ink.

Well No. 1 is marked with the Lot No. '18F'.

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

Please note: When removing each 8 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Only the DRB1*09 alleles will be amplified by the DRB1*09 subtyping kit. Thus, the interpretation of DRB1*09 subtypings is not influenced by other groups of DRB1 alleles or other DRB genes.

UNIQUELY IDENTIFIED ALLELES

All the DRB1*09 alleles, i.e. **DRB1*0901 to DRB1*0907**, recognized by the HLA Nomenclature Committee in July 2008¹ will give rise to unique amplification patterns by the primers in the DRB1*09 subtyping kit.

The DRB1*09 SSP subtyping kit cannot separate the DRB1*090102 to DRB1*090104 alleles.

¹DRB alleles listed on the IMGT/HLA web page 2008-July-11, release 2.22.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The 7 DRB1*09 alleles can be combined in 28 homozygous and heterozygous combinations. Eight of these genotypes do not give rise to unique amplification patterns.

+++----- 0901,0903 = 0903,0903
+++----- 0901,0904 = 0904,0904
+-----+- 0901,0906 = 0906,0906
+-----+ 0901,0907 = 0907,0907

0901 = 090102-090104

SPECIFICITY TABLE

DRB1*09 SSP subtyping

Specificities and sizes of the PCR products of the 8 primer mixes used for DRB1*09 SSP subtyping

| Primer Mix | Size of spec. PCR product ¹ | Size of control band ² | Amplified DRB1*09 alleles |
|------------|--|-----------------------------------|---------------------------|
| 1 | 195 bp | 515 bp | 090102-0904, 0906, 0907 |
| 2 | 130 bp | 430 bp | 090102-090104, 0903-0907 |
| 3 | 135 bp | 430 bp | 0902 |
| 4 | 215 bp | 430 bp | 0903 |
| 5 | 215 bp | 430 bp | 0904 |
| 6 | 250 bp | 430 bp | 0905 |
| 7 | 220 bp | 430 bp | 0906 |
| 8 | 175 bp | 430 bp | 0907 |

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 DRB1*09 SSP typings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 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*09 subtyping.

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

| INTERPRETATION TABLE | | | | | | | | |
|--|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| DRB1*09 SSP subtyping | | | | | | | | |
| Amplification patterns of the DRB1*09 alleles | | | | | | | | |
| | Well | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Length of spec. | 195 | 130 | 135 | 215 | 215 | 250 | 220 | 175 |
| PCR product | | | | | | | | |
| Length of int. | 515 | 430 | 430 | 430 | 430 | 430 | 430 | 430 |
| pos. control¹ | | | | | | | | |
| 5'-primer² | 26 | 28 | 26 | 25 | 25 | 25 | 26 | 13 |
| | 5'-g TA ^{3'} | 5'-g CA ^{3'} | 5'-g TA ^{3'} | 5'-Tg A ^{3'} | 5'-Tg A ^{3'} | 5'-Tg A ^{3'} | 5'-TAT ^{3'} | 5'-g TA ^{3'} |
| 3'-primer³ | 78 | 57 | 57 | 67 | 67 | 78 | 86 | 57 |
| | 5'-CAC ^{3'} | 5'-C gA ^{3'} | 5'-gC g ^{3'} | 5'-gAA ^{3'} | 5'-gAg ^{3'} | 5'-gTA ^{3'} | 5'-C CA ^{3'} | 5'-C gA ^{3'} |
| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| DRB1 allele⁴ | | | | | | | | |
| *090102-090104 | 1 | 2 | | | | | | |
| *0902 | 1 | | 3 | | | | | |
| *0903 | 1 | 2 | | 4 | | | | |
| *0904 | 1 | 2 | | | 5 | | | |
| *0905 | | 2 | | | | 6 | | |
| *0906 | 1 | 2 | | | | | 7 | |
| *0907 | 1 | 2 | | | | | | 8 |
| DRB1 allele⁴ | | | | | | | | |
| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

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*09 subtyping.

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

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

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

⁴The nucleotide sequence of the DRB1*090101 allele has been shown to contain errors and be identical to DRB1*090102.

| CELL LINE VALIDATION SHEET | | | | | | | | | | | | |
|----------------------------|----------------|--|-------|----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| DRB1*09 SSP subtyping kit | | | | | | | | | | | | |
| | | | | Production No. | Well | | | | | | | |
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| | | | | | 200616901 | 200616902 | 200616903 | 200616904 | 200616905 | 200616906 | 200616907 | 200852208 |
| | IHWC cell line | | DRB1 | | | | | | | | | |
| 1 | 9001 SA | | *0101 | | - | - | - | - | - | - | - | - |
| 2 | 9280 LK707 | | *1502 | *0405 | - | - | - | - | - | - | - | - |
| 3 | 9011 E4181324 | | *1502 | | - | - | - | - | - | - | - | - |
| 4 | 9275 GU373 | | *0301 | | - | - | - | - | - | - | - | - |
| 5 | 9009 KAS011 | | *1601 | | - | - | - | - | - | - | - | - |
| 6 | 9353 SM | | *0407 | *0803 | - | - | - | - | - | - | - | - |
| 7 | 9020 QBL | | *0301 | | - | - | - | - | - | - | - | - |
| 8 | 9007 DEM | | *0401 | *1602 | - | - | - | - | - | - | - | - |
| 9 | 9026 YAR | | *0402 | | - | - | - | - | - | - | - | - |
| 10 | 9107 LKT3 | | *0405 | | - | - | - | - | - | - | - | - |
| 11 | 9051 PITOUT | | *0701 | | - | - | - | - | - | - | - | - |
| 12 | 9052 DBB | | *0701 | | - | - | - | - | - | - | - | - |
| 13 | 9067 BTB | | *0801 | | - | - | - | - | - | - | - | - |
| 14 | 9071 OLGA | | *0802 | | - | - | - | - | - | - | - | - |
| 15 | 9075 DKB | | *0901 | | + | + | - | - | - | - | - | - |
| 16 | 9037 SWEIG007 | | *1101 | | - | - | - | - | - | - | - | - |
| 17 | 9008 WILJON | | *1501 | | - | - | - | - | - | - | - | - |
| 18 | 9257 32367 | | *0901 | *1101 | + | + | - | - | - | - | - | - |
| 19 | 9038 BM16 | | *1201 | | - | - | - | - | - | - | - | - |
| 20 | 9059 SLE005 | | *1302 | | - | - | - | - | - | - | - | - |
| 21 | 9064 AMALA | | *1402 | | - | - | - | - | - | - | - | - |
| 22 | 9056 KOSE | | *1302 | *1401 | - | - | - | - | - | - | - | - |
| 23 | 9124 IHL | | *0803 | *1414 | - | - | - | - | - | - | - | - |
| 24 | 9035 JBUSH | | *1101 | | - | - | - | - | - | - | - | - |
| 25 | 9049 IBW9 | | *0701 | | - | - | - | - | - | - | - | - |
| 26 | 9285 WT49 | | *0301 | | - | - | - | - | - | - | - | - |
| 27 | 9191 CH1007 | | *0405 | *1001 | - | - | - | - | - | - | - | - |
| 28 | 9320 BEL5GB | | *0416 | *0701 | - | - | - | - | - | - | - | - |
| 29 | 9050 MOU | | *0701 | | - | - | - | - | - | - | - | - |
| 30 | 9021 RSH | | *0302 | | - | - | - | - | - | - | - | - |
| 31 | 9019 DUCAF | | *0301 | | - | - | - | - | - | - | - | - |
| 32 | 9297 HAG | | *1303 | | - | - | - | - | - | - | - | - |
| 33 | 9098 MT14B | | *0404 | | - | - | - | - | - | - | - | - |
| 34 | 9104 DHIF | | *1101 | | - | - | - | - | - | - | - | - |
| 35 | 9302 SSTO | | *0403 | | - | - | - | - | - | - | - | - |
| 36 | 9024 KT17 | | *0403 | *0406 | - | - | - | - | - | - | - | - |
| 37 | 9065 HHKB | | *1301 | | - | - | - | - | - | - | - | - |
| 38 | 9099 LZL | | *1402 | | - | - | - | - | - | - | - | - |
| 39 | 9315 CML | | *0301 | *0401 | - | - | - | - | - | - | - | - |
| 40 | 9134 WHONP199 | | *0701 | *0901 | + | + | - | - | - | - | - | - |
| 41 | 9055 H0301 | | *1302 | | - | - | - | - | - | - | - | - |
| 42 | 9066 TAB089 | | *0803 | | - | - | - | - | - | - | - | - |
| 43 | 9076 T7526 | | *0901 | | + | + | - | - | - | - | - | - |
| 44 | 9057 TEM | | *1401 | | - | - | - | - | - | - | - | - |
| 45 | 9239 SHJO | | *0701 | | - | - | - | - | - | - | - | - |
| 46 | 9013 SCHU | | *1501 | | - | - | - | - | - | - | - | - |
| 47 | 9045 TUBO | | *1104 | *1201 | - | - | - | - | - | - | - | - |
| 48 | 9303 TER-ND | | *0103 | | - | - | - | - | - | - | - | - |

CERTIFICATE OF ANALYSIS

Olerup SSP[®] DRB1*09 SSP

Product number: 101.120-06u – without *Taq* polymerase
Lot number: 18F
Expiry date: 2010-October-01
Number of tests: 6
Number of wells per test: 8

Well specifications:

| Well No. | Production No. |
|----------|----------------|
| 1 | 2006-169-01 |
| 2 | 2006-169-02 |
| 3 | 2006-169-03 |
| 4 | 2006-169-04 |
| 5 | 2006-169-05 |
| 6 | 2006-169-06 |
| 7 | 2006-169-07 |
| 8 | 2008-522-08 |

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

No DNAs carrying the allele to be amplified by primer solutions 3, 4, 5, 6, 7 and 8 were available. The specificities of the primers in primer solutions 3, 4, 5, 6, 7 and 8 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer.

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

Date of approval: 2009-May-25

Approved by:

Quality Control, Supervisor

Declaration of Conformity

Product name: *Olerup* SSP® DRB1*09
Product number: 101.120-06u
Lot number: 18F

Intended use: DRB1*09 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2000 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, conformity assessed using Annex IV, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

The Authorized Representative located within the Community is: *Olerup* SSP AB.

Notified Body: Lloyd's Register Quality Assurance Limited, Hiramford, Middlemarch Office Village, Siskin Drive, Coventry CV3 4FJ, United Kingdom. (Notified Body number: 0088.)

Saltsjöbaden, Sweden
2009-May-25

Olle Olerup
Managing Director

Lot No.: **18F**

Lot-specific Information

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