

Lot No.: **84F**

Lot-specific Information

***Olerup SSP*[®] HLA-B*47**

| | |
|----------------------------------|---|
| Product number: | 101.545-06u – without <i>Taq</i> polymerase |
| Lot number: | 84F |
| Expiry date: | 2011-May-01 |
| Number of tests: | 6 |
| Number of wells per test: | 7 |
| 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. 84F.

CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*[®] HLA-B*47 LOT.

The HLA-B*47 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP*[®] HLA-B*47 lot was made (**Lot No. 13E**).

The HLA-B*47 primer set is unchanged compared to the previous lot (**Lot No. 13E**).

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PRODUCT DESCRIPTION

HLA-B*47 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*4701 to B*4705 alleles.

PLATE LAYOUT

Each HLA-B*47 test consists of 7 PCR reactions in an 8 well cut PCR plate. Well 8 is empty.

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

The 8 well PCR plate is marked with 'B47' in silver/gray ink.

Well No. 1 is marked with the Lot No. '84F'.

The PCR plates are heat-sealed with a PCR-compatible foil.

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

INTERPRETATION

The interpretation of HLA-B*47 SSP subtypings will be influenced by ten B*15, the B*1827 allele, most B*27, three B*35, two B*37, most B*40, the B*41, most B*44, the B*45, five B*49, the B*50 and the B*82 alleles when present on the other haplotype.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*47, i.e. **B*4701 to B*4705**, recognized by the HLA Nomenclature Committee in May 2009¹ will be amplified by the primers in the HLA-B*47 SSP kit. The HLA-B*47 kit cannot distinguish the B*47010101 and B*47010102 alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2009-May-11, release 2.25.2, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

The five HLA-B*47 alleles can be combined in 15 homozygous and heterozygous combinations. All these genotypes give rise to unique amplification patterns.

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SPECIFICITY TABLE

HLA-B*47 SSP subtyping

Specificities and sizes of the PCR products of the 7 primer mixes used for HLA-B*47 SSP subtyping

| Primer Mix | Size of spec. PCR product ¹ | Size of control band ² | Amplified HLA-B*47 alleles | Other amplified HLA-B alleles ³ |
|------------|--|-----------------------------------|------------------------------|---|
| 1 | 135 bp | 800 bp | 47010101-47010102, 4705 | 3706 |
| 2 | 150 bp | 1070 bp | 47010101-47010102, 4703-4705 | 3706, 4013, 4019, 4047, 4096, 44020101-4405, 4407, 4410-4439, 4441-4443, 4445, 4447-4456N, 4458N, 4459, 4461N-4467, 4469, 490101-4902, 4904, 4905 |
| 3 | 150 bp | 1070 bp | 4702 | 1546, 1553, 9506, 9543, 3519, 3547, 3563, 400101-4011, 401401-4016, 4018, 4020, 4022N-4040, 4042-4045, 4048-4078, 4080-4092, 4094, 4095, 4101-4108, 4409, 4446, 4501-4509, 5001, 5002, 5004 |
| 4 | 145 | 1070 bp | 4704 | 4047, 4096, 44020101-4405, 4407, 4410, 4412-4417, 4419N-4424, 4426-4439, 4441-4443, 4445, 4447-4449, 4451-4456N, 4458N, 4459, 4461N-4467, 4469, 4902 |
| 5 | 135 bp | 1070 bp | 4702, 4703 | 1546, 1553, 9506, 9543, 3519, 3547, 3563, 400101-4011, 401401-4016, 4018, 4020, 4022N-4036, 4038-4040, 4042- |

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| | | | | |
|----------|--------|---------|---------------|---|
| | | | | 4045, 4048-4092, 4094, 4095, 4101-4108, 4409, 4446, 4501-4509, 5001, 5002, 5004 |
| 6 | 135 bp | 1070 bp | 47010101-4703 | 1506, 152701-152703, 1584, 9509, 1827, 8201, 8202 |
| 7 | 235 bp | 1070 bp | 4704, 4705 | 2701-270402, 270502-2706, 2708-2710, 2712, 2713, 2716-2718, 2720, 2723, 2726, 2727, 2729, 2731, 2735-2737, 2739-2742, 2744-2746, 2750, 3702 |

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 HLA-B*47 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 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*47 SSP subtyping.

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

³Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B*47 alleles will be amplified by primer mixes 1 to 7.

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| HLA-B*47 SSP subtyping | | | | | | | |
|---|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Amplification patterns of the B*4701 to 4705 alleles | | | | | | | |
| | Well | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Length of spec. | 135 | 150 | 150 | 145 | 135 | 135 | 235 |
| PCR product | | | | | | | |
| Length of int. | 800 | 1070 | 1070 | 1070 | 1070 | 1070 | 1070 |
| pos. control ¹ | | | | | | | |
| 5'-primer ² | 206 | 206 | 206 | 206 | 206 | 368 | 363 |
| | 5'-gAA ^{3'} | 5'-gAA ^{3'} | 5'-gAA ^{3'} | 5'-gAA ^{3'} | 5'-gAA ^{3'} | 5'-gTT ^{3'} | 5'-AAT ^{3'} |
| 3'-primer ³ | 301 | 317 | 317 | 309 | 301 | 463 | 559 |
| | 5'-gTC ^{3'} | 5'-ggA ^{3'} | 5'-CgC ^{3'} | 5'-gTg ^{3'} | 5'-gCT ^{3'} | 5'-gCT ^{3'} | 5'-CTC ^{3'} |
| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| HLA-B allele | | | | | | | |
| *47010101-47010102 | 1 | 2 | | | | 6 | |
| *4702 | | | 3 | | 5 | 6 | |
| *4703 | | 2 | | | 5 | 6 | |
| *4704 | | 2 | | 4 | | | 7 |
| *4705 | 1 | 2 | | | | | 7 |
| *1506, 152701-152703, 1584, 9509, 1827, 8201, 8202 | | | | | | 6 | |
| *1546, 1553, 9506, 9543, 3519, 3547, 3563, 400101-4011, 401401-4016, 4018, 4020, 4022N-4036, 4038-4040, 4042-4045, 4048-4078, 4080-4092, 4094, 4095, 4101-4108, 4409, 4446, 4501-4509, 5001, 5002, 5004 | | | 3 | | 5 | | |
| *2701-270402, 270502-2706, 2708-2710, 2712, 2713, 2716-2718, 2720, 2723, 2726, 2727, 2729, 2731, 2735-2737, 2739-2742, 2744-2746, 2750, 3702 | | | | | | | 7 |
| *3706 | 1 | 2 | | | | | |
| *4013, 4019, 4411, 4418, 4425, 4450, 490101-490102, 4904, 4905 | | 2 | | | | | |
| *4037 | | | 3 | | | | |
| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

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| Length of spec. | 135 | 150 | 150 | 145 | 135 | 135 | 235 |
|---|-----|-----|-----|-----|-----|-----|-----|
| PCR product | | | | | | | |
| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| *4047, 4096, 44020101-4405, 4407, 4410, 4412-4417, 4419N-4424, 4426-4439, 4441-4443, 4445, 4447-4449, 4451-4456N, 4458N, 4459, 4461N-4467, 4469, 4902 | | 2 | | 4 | | | |
| *4079 | | | | | 5 | | |
| HLA-B allele | | | | | | | |
| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

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 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-B*47 SSP subtyping.

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

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

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Lot-specific Information

| CELL LINE VALIDATION SHEET | | | | | | | | | | | |
|----------------------------|-----------------|--|--------|------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| HLA-B*47 SSP primer set | | | | | | | | | | | |
| | | | | | Well | | | | | | |
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | | | | Prod. No.: | 200959201 | 200959202 | 200959203 | 200959204 | 200959205 | 200959206 | 200959207 |
| | IHWC cell line | | B* | | | | | | | | |
| 1 | 9001 SA | | *0702 | | - | - | - | - | - | - | - |
| 2 | 9280 LK707 | | *5201 | *7301 | - | - | - | - | - | - | - |
| 3 | 9011 E4181324 | | *52011 | | - | - | - | - | - | - | - |
| 4 | 9275 GU373 | | *1510 | *5301 | - | - | - | - | - | - | - |
| 5 | 9009 KAS011 | | *3701 | | - | - | - | - | - | - | - |
| 6 | 9353 SM | | *3901 | *5101 | - | - | - | - | - | - | - |
| 7 | 9020 QBL | | *1801 | | - | - | - | - | - | - | - |
| 8 | 9025 DEU | | *3501 | | - | - | - | - | - | - | - |
| 9 | 9026 YAR | | *3801 | | - | - | - | - | - | - | - |
| 10 | 9107 LKT3 | | *5401 | | - | - | - | - | - | - | - |
| 11 | 9051 PITOUT | | *4403 | | - | + | - | + | - | - | - |
| 12 | 9052 DBB | | *5701 | | - | - | - | - | - | - | - |
| 13 | 9004 JESTHOM | | *2705 | | - | - | - | - | - | - | + |
| 14 | 9071 OLGA | | *1501 | *1520 | - | - | - | - | - | - | - |
| 15 | 9075 DKB | | *4001 | | - | - | + | - | + | - | - |
| 16 | 9037 SWEIG007 | | *4002 | | - | - | + | - | + | - | - |
| 17 | 9282 CTM3953540 | | *0801 | *5501 | - | - | - | - | - | - | - |
| 18 | 9257 32367 | | *1401 | *5601 | - | - | - | - | - | - | - |
| 19 | 9038 BM16 | | *1801 | | - | - | - | - | - | - | - |
| 20 | 9059 SLE005 | | *4001 | | - | - | + | - | + | - | - |
| 21 | 9064 AMALA | | *1501 | | - | - | - | - | - | - | - |
| 22 | 9056 KOSE | | *3503 | | - | - | - | - | - | - | - |
| 23 | 9124 IHL | | *4002 | *5602 | - | - | + | - | + | - | - |
| 24 | 9035 JBUSH | | *3801 | | - | - | - | - | - | - | - |
| 25 | 9049 IBW9 | | *1402 | | - | - | - | - | - | - | - |
| 26 | 9285 WT49 | | *5801 | | - | - | - | - | - | - | - |
| 27 | 9191 CH1007 | | *0705 | *5101 | - | - | - | - | - | - | - |
| 28 | 9320 BEL5GB | | *4402 | *4403 | - | + | - | + | - | - | - |
| 29 | 9050 MOU | | *4403 | | - | + | - | + | - | - | - |
| 30 | 9021 RSH | | *4201 | | - | - | - | - | - | - | - |
| 31 | 9019 DUCAF | | *1801 | | - | - | - | - | - | - | - |
| 32 | 9297 HAG | | *4102 | | - | - | + | - | + | - | - |
| 33 | 9098 MT14B | | *4001 | | - | - | + | - | + | - | - |
| 34 | 9104 DHIF | | *3801 | | - | - | - | - | - | - | - |
| 35 | 9302 SSTO | | *4402 | | - | + | - | + | - | - | - |
| 36 | 9024 KT17 | | *1501 | *3501 | - | - | - | - | - | - | - |
| 37 | 9065 HHKB | | *0702 | | - | - | - | - | - | - | - |
| 38 | 9099 LZL | | *1501 | | - | - | - | - | - | - | - |
| 39 | 9315 CML | | *0801 | *2705 | - | - | - | - | - | - | + |
| 40 | 9134 WHONP199 | | *1302 | *4601 | - | - | - | - | - | - | - |
| 41 | 9055 H0301 | | *1402 | | - | - | - | - | - | - | - |
| 42 | 9066 TAB089 | | *4601 | | - | - | - | - | - | - | - |
| 43 | 9076 T7526 | | *4601 | | - | - | - | - | - | - | - |
| 44 | 9057 TEM | | *3801 | | - | - | - | - | - | - | - |
| 45 | 9239 SHJO | | *4201 | *5001 | - | - | + | - | + | - | - |
| 46 | 9013 SCHU | | *0702 | | - | - | - | - | - | - | - |
| 47 | 9045 TUBO | | *5101 | | - | - | - | - | - | - | - |
| 48 | 9303 TER-ND | | *3501 | *4403 | - | + | - | + | - | - | - |

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CERTIFICATE OF ANALYSIS

Olerup SSP[®] HLA-B*47 SSP

Product number: 101.545-06u – without *Taq* polymerase
Lot number: 84F
Expiry date: 2011-May-01
Number of tests: 6
Number of Wells per test: 7

Well specifications:

| Well No. | Production No. |
|----------|----------------|
| 1 | 2009-592-01 |
| 2 | 2009-592-02 |
| 3 | 2009-592-03 |
| 4 | 2009-592-04 |
| 5 | 2009-592-05 |
| 6 | 2009-592-06 |
| 7 | 2009-592-07 |

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

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

Date of approval: 2009-May-27

Approved by:

Quality Control, Supervisor

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Declaration of Conformity

Product name: *Olerup* SSP® HLA-B*47
Product number: 101.545-06u
Lot number: 84F

Intended use: HLA-B*47 high resolution histocompatibility testing

Manufacturer: *Olerup* SSP AB
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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, 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-27

Olle Olerup
Managing Director

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