

## Olerup SSP<sup>®</sup> HLA-B\*81

|                                  |  |
|----------------------------------|--|
| Product number:                  | 101.553-06 – including <i>Taq</i> polymerase |
| Lot number:                      | 56F  |
| Expiry date:                     | 2011-February-01                             |
| Number of tests:                 | 6  |
| Number of wells per test:        | 6  |
| 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. 56F.**

### CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*<sup>®</sup> HLA-B\*81 LOT.

The HLA-B\*81 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup SSP*<sup>®</sup> HLA-B\*81 lot was made (Lot No. 18E).

One well has been added to the HLA-B\*81 kit,  
well **6**.

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

| Well | 5'-primer | 3'-primer | rationale   |
|------|-----------|-----------|---|
| 3    | -         | Exchanged | New 3'-primer to increase specificity of amplification.               |
| 5    | Exchanged | -         | New 5'-primer to improve resolution of the B*8101 and B*8103 alleles. |
| 6    | New       | New       | New primer pair for the B*8104N allele.                               |

## PRODUCT DESCRIPTION

### HLA-B\*81 SSP typing

#### CONTENT

The primer set contains 5'- and 3'-primers for separating the B\*8101 to B\*8104N alleles.

#### PLATE LAYOUT

Each HLA-B\*81 test consists of 6 PCR reactions in an 8 well cut PCR plate. Wells 7 and 8 are empty.

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

The 8 well PCR plate is marked with 'B81' in silver/gray ink.

Well No. 1 is marked with the Lot No. '56F'.

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\*81 SSP subtypings will be influenced by most B\*07, most B\*08, the B\*1520, most B\*27, most B\*35, the B\*3707, six B\*40, two B\*41, the B\*42, the B\*4506, the B\*48, the B\*51, the B\*52, the B\*53, the B\*54, the B\*55, the B\*56, the B\*58, the B\*6701, the B\*78, the B\*82 and the B\*8301 alleles when present on the other haplotype.

#### UNIQUELY IDENTIFIED ALLELES

All the HLA-B\*81 alleles, i.e. **B\*8101 to B\*8104N**, recognized by the HLA Nomenclature Committee in January 2009<sup>1</sup> will be amplified by the primers in the HLA-B\*81 SSP kit.

<sup>1</sup>HLA-B alleles listed on the IMGT/HLA web page 2009-January-16, release 2.24.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

#### RESOLUTION IN HOMO- AND HETEROZYGOTES

The 4 HLA-B\*81 alleles can be combined in 10 homozygous and heterozygous combinations. Six of these genotypes do not give rise to unique amplification patterns.

+++++ 8101,8103 = 8102,8103 = 8103,8103  
+++++ 8101,8104N = 8102,8104N = 8104N,8104N

## SPECIFICITY TABLE

### HLA-B\*81 SSP subtyping

Specificities and sizes of the PCR products of the 6 primer mixes used for HLA-B\*81 SSP subtyping

| Primer Mix     | Size of spec. PCR product <sup>1</sup> | Size of control band <sup>2</sup> | Amplified HLA-B*81 alleles                   | Other amplified HLA-B alleles <sup>3</sup>   |
|----------------|--|-----------------------------------|--|--|
| 1              | 175 bp                                 | 800 bp                            | 8101-8104N                                   | 2724, 4031, 4045, 4080, 480101-480102, 4804, 4806, 4807, 4809, 4811, 4815, 4816, 4818, 4819  |
| 2              | 410 bp                                 | 1070 bp                           | 8101, 8103 <sup>?</sup> , 8104N <sup>?</sup> | 2702-270402, 270502-2711, 2713-2715, 2717, 2719-2721, 2724-2728, 2730-2738, 2740-2745, 3576, 5401-5417, 550101-5505, 5507-5517, 5519-5534, 5601-5616, 5618-5622, 5624, 8201, 8202, 8301                        |
| 3 <sup>4</sup> | 410 bp                                 | 1070 bp                           | 8102, 8103 <sup>?</sup> , 8104N <sup>?</sup> | 070201-070207, 0704-0707, 0709- 0715, 0717-0726, 0728-0731, 0733-0736, 0739-0746, 0748, 0749N, 0751-0764, 0766-0768, 4201, 4202, 4204-4206, 4208, 4209, 4506, 670101-6702, 7301                                |
| 4              | 285 bp                                 | 1070 bp                           | 8101-8104N <sup>?</sup>                      | 070201-0706, 0708-0711, 0713, 0715-0717, 0720-0732, 0734-0739, 0741-0752, 0754-0759, 0761-0768, 3566, 3707, 4015, 4016, 4031, 4032, 4045, 4080, 420501-420502, 480101-480102, 4805-4812, 4814-4816, 4818, 4819 |
| 5              | 165 bp                                 | 1070 bp                           | 8103   | 350101-3530, 3532-   |

|          |       |         |       |  |
|----------|-------|---------|-------|--|
|          |       |         |       | 3542, 3544, 3545,<br>3547-3556, 3558,<br>3559, 3561-3572,<br>3574-3578, 3580-3586,<br>3588-3594, 4802,<br>510101-512402, 5126-<br>5141N, 5143-5146,<br>5148-5163, 520101-<br>520602, 5208-5214,<br>530101-5316, 580101-<br>5802, 5804-5810N,<br>5812-5819, 7801-7806 |
| <b>6</b> | 80 bp | 1070 bp | 8104N |  |

<sup>1</sup>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 HLA-B\*81 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.

<sup>2</sup>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 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 the presence of a specific amplification the intensity of the control band often decreases.

<sup>3</sup>Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B\*81 alleles will be amplified by primer mixes 1 to 5.

<sup>4</sup>Primer mix 3 may give rise to a primer oligomer artifact.

| <b>INTERPRETATION TABLE</b>   |                                    |                                    |                                    |                                    |                                    |                                    |
|---|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| <b>HLA-B*81 SSP subtyping</b>   |                                    |                                    |                                    |                                    |                                    |                                    |
| <b>Amplification patterns of the B*81 alleles</b>   |                                    |                                    |                                    |                                    |                                    |                                    |
|   | <b>Well</b>                        |                                    |                                    |                                    |                                    |                                    |
|   | <b>1</b>                           | <b>2</b>                           | <b>3</b>                           | <b>4</b>                           | <b>5</b>                           | <b>6</b>                           |
| <b>Length of spec.</b>  | <b>175</b>                         | <b>410</b>                         | <b>410</b>                         | <b>285</b>                         | <b>165</b>                         | <b>80</b>                          |
| <b>PCR product</b>  |                                    |                                    |                                    |                                    |                                    |                                    |
| <b>Length of int.</b>   | <b>800</b>                         | <b>1070</b>                        | <b>1070</b>                        | <b>1070</b>                        | <b>1070</b>                        | <b>1070</b>                        |
| <b>pos. control<sup>1</sup></b>   |                                    |                                    |                                    |                                    |                                    |                                    |
| <b>5'-primer<sup>2</sup></b>  | <b>363</b>                         | <b>41</b>                          | <b>44</b>                          | <b>363</b>                         | <b>652</b>                         | <b>206</b>                         |
|   | <small>5'-AgC<sup>3'</sup></small> | <small>5'-CTg<sup>3'</sup></small> | <small>5'-ggC<sup>3'</sup></small> | <small>5'-AgC<sup>3'</sup></small> | <small>5'-CCg<sup>3'</sup></small> | <small>5'-AgA<sup>3'</sup></small> |
| <b>3'-primer<sup>3</sup></b>  | <b>499</b>                         | <b>282</b>                         | <b>282</b>                         | <b>605</b>                         | <b>774</b>                         | <b>244</b>                         |
|   | <small>5'-ggA<sup>3'</sup></small> | <small>5'-gCC<sup>3'</sup></small> | <small>5'-gCC<sup>3'</sup></small> | <small>5'-gCT<sup>3'</sup></small> | <small>5'-ggT<sup>3'</sup></small> | <small>5'-CTA<sup>3'</sup></small> |
| <b>Well No.</b>   | <b>1</b>                           | <b>2</b>                           | <b>3</b>                           | <b>4</b>                           | <b>5</b>                           | <b>6</b>                           |
| <b>HLA-B allele</b>   |                                    |                                    |                                    |                                    |                                    |                                    |
| <b>*8101</b>  | <b>1</b>                           | <b>2</b>                           |                                    | <b>4</b>                           |                                    |                                    |
| <b>*8102</b>  | <b>1</b>                           |                                    | <b>3</b>                           | <b>4</b>                           |                                    |                                    |
| <b>*8103</b>  | <b>1</b>                           | <b>?</b>                           | <b>?</b>                           | <b>4</b>                           | <b>5</b>                           |                                    |
| <b>*8104N</b>   | <b>1</b>                           | <b>?</b>                           | <b>?</b>                           | <b>4</b>                           |                                    | <b>6</b>                           |
| <b>*070201-070207, 0704-0706, 0709-0711, 0713, 0715, 0717, 0720-0726, 0728-0731, 0734-0736, 0739, 0741-0746, 0748, 0749N, 0751, 0752, 0754-0759, 0761-0764, 0766-0768, 420501-420502</b>  |                                    |                                    | <b>3</b>                           | <b>4</b>                           |                                    |                                    |
| <b>*0703, 0708, 0716, 0727, 0732, 0737, 0738, 0747, 0750, 0765, 3707, 4015, 4016, 4032, 4805, 4808, 4810, 4812, 4814</b>  |                                    |                                    |                                    | <b>4</b>                           |                                    |                                    |
| <b>*0707, 0712, 0714, 071801-0719, 0733, 0740, 0753, 0760, 4201, 4202, 4204, 4206, 4208, 4209, 4506, 670101-6702, 7301</b>  |                                    |                                    | <b>3</b>                           |                                    |                                    |                                    |
| <b>*2702-270402, 270502-2711, 2713-2715, 2717, 2719-2721, 2725-2728, 2730-2738, 2740-2745, 5401-5417, 550101-5505, 5507-5517, 5519-5534, 5601-5616, 5618-5622, 5624, 8201, 8202, 8301</b> |                                    | <b>2</b>                           |                                    |                                    |                                    |                                    |
| <b>Well No.</b>   | <b>1</b>                           | <b>2</b>                           | <b>3</b>                           | <b>4</b>                           | <b>5</b>                           | <b>6</b>                           |

| Length of spec.  | 175 | 410 | 410 | 285 | 165 | 80 |
|--|-----|-----|-----|-----|-----|----|
| PCR product  |     |     |     |     |     |    |
| Well No.   | 1   | 2   | 3   | 4   | 5   | 6  |
| *2724  | 1   | 2   |     |     |     |    |
| *350101-3530, 3532-3542, 3544, 3545, 3547-3556, 3558, 3559, 3561-3565Q, 3567-3572, 3574, 3575, 3577, 3578, 3580-3586, 3588-3594, 510101-512402, 5126-5141N, 5143-5146, 5148-5163, 520101-520602, 5208-5214, 530101-5316, 580101-5802, 5804-5810N, 5812-5819, 7801-7806 |     |     |     |     | 5   |    |
| *3566  |     |     |     | 4   | 5   |    |
| *3576  |     | 2   |     |     | 5   |    |
| *4031, 4045, 4080, 480101-480102, 4806, 4807, 4809, 4811, 4815, 4816, 4818, 4819   | 1   |     |     | 4   |     |    |
| *4804  | 1   |     |     |     |     |    |
| HLA-B allele   |     |     |     |     |     |    |
| Well No.   | 1   | 2   | 3   | 4   | 5   | 6  |

<sup>1</sup>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 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.

<sup>2</sup>The nucleotide position, in the 1<sup>st</sup>, 2<sup>nd</sup> or 3<sup>rd</sup> exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>3</sup>The nucleotide position, in the 2<sup>nd</sup> or 3<sup>rd</sup> 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](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

| CELL LINE VALIDATION SHEET |      |            |             |            |           |           |           |           |           |           |
|----------------------------|------|------------|-------------|------------|-----------|-----------|-----------|-----------|-----------|-----------|
| HLA-B*81 SSP primer set    |      |            |             |            |           |           |           |           |           |           |
|                            |      |            |             | Well       |           |           |           |           |           |           |
|                            |      |            |             | 1          | 2         | 3         | 4         | 5         | 6         |           |
|                            |      |            |             | Prod. No.: | 200956101 | 200956102 | 200956103 | 200956104 | 200956105 | 200956106 |
| IHC 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                          | 9007 | DEM        | *5701       | -          | -         | -         | -         | -         | -         |           |
| 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 | -          | -         | -         | -         | +         | -         |           |

## CERTIFICATE OF ANALYSIS

### Olerup SSP® HLA-B\*81 SSP

Product number: 101.553-06 – including Taq polymerase  
Lot number: 56F  
Expiry date: 2011-February-01  
Number of tests: 6  
Number of wells per test: 6

#### Well specifications:

| Well No. | Production No. |
|----------|----------------|
| 1        | 2009-561-01    |
| 2        | 2009-561-02    |
| 3        | 2009-561-03    |
| 4        | 2009-561-04    |
| 5        | 2009-561-05    |
| 6        | 2009-561-06    |

The specificity of each primer solution of the HLA-B\*81 primer set has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solution 6 were available. The specificities of the primers in this primer solution was tested by separately adding one additional 3'-primer, the 3'-primer was not possible to test.

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

**Date of approval:** 2009-May-27

**Approved by:**

**Quality Control, Supervisor**



Lot No.: **56F**

Lot-specific information

[www.olerup.com](http://www.olerup.com)

## Declaration of Conformity

**Product name:** *Olerup SSP*<sup>®</sup> HLA-B\*81  
**Product number:** 101.553-06  
**Lot number:** 56F

**Intended use:** HLA-B\*81 high resolution histocompatibility testing

**Manufacturer:** *Olerup SSP AB*  
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**Phone:** +46-8-717 88 27  
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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-27

Olle Olerup  
Managing Director





Lot No.: **56F**

Lot-specific information

[www.olerup.com](http://www.olerup.com)

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