

101.555-06 – including *Taq* pol., IFU-01
101.555-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **92V**

Lot-specific information
Olerup SSP® HLA-B*73

www.olerup-ssp.com

| | |
|---|---|
| Product number: | 101.555-06 – including <i>Taq</i> polymerase 101.555-06u – without <i>Taq</i> polymerase |
| Lot number: | 92V |
| Expiry date: | 2016-November-01 |
| Number of tests: | 6 |
| Number of wells per test: | 3+1 |
| 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. 92V.

Complete product documentation consists of generic Instructions for Use (IFU), lot specific Product Insert, Worksheet and Certificate.

**CHANGES COMPARED TO THE PREVIOUS OLERUP SSP®
HLA-B*73 Lot (11R)**

The HLA-B*73 kit is updated for new alleles to enable separation of:

- Confirmed¹ alleles as listed in the IMGT/HLA database
- Polymorphisms in exons outside of the region encoding the peptide binding domain
- Null and Alternatively expressed alleles

A well containing Negative Control primer pairs has been added.

The format of the Product Insert and Worksheet have been changed.

One well has been added to HLA-B*73, well **4**.

¹As described in section Uniquely Identified Alleles.

The HLA-B*73 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP®* HLA-B*73 lot was made (**Lot No. 11R**).

101.555-06 – including *Taq* pol., IFU-01
101.555-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **92V**

Lot-specific information

www.olerup-ssp.com

As of lot series V, the Specificity Table is included in the lot-specific Product Insert, and the Interpretation Table is included in the Worksheet.

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

| Well | 5'-primer | 3'-primer | rationale |
|------|-----------|-----------|-------------------|
| 4 | - | - | Negative Control. |

101.555-06 – including *Taq* pol., IFU-01
101.555-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **92V**

Lot-specific information

www.olerup-ssp.com

Well 4 contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup SSP®* HLA Class I, DRB, DQB1 and DPB1 amplicons as well as amplicons generated by a control primer pair.

PCR product sizes range from 75 to 430 base pairs.
The PCR product generated by the control primer pair is 430 base pairs.

| Length of PCR product | 105 | 200 | 105 | 80 | 75 | 80 |
|------------------------------|----------------------|-------------------------|----------------------|----------------------|----------------------|----------------------|
| 5'-primer¹ | 164 | 340 | 440 | 45 | 45 | 43 |
| | 5'-CAC ^{3'} | 5'-Agg ^{3'} | 5'-TTA ^{3'} | 5'-Tgg ^{3'} | 5'-Tgg ^{3'} | 5'-Tgg ^{3'} |
| 3'-primer² | 231 | 2nd I | 507 | 59 | 58 | 57 |
| | 5'-TgC ^{3'} | 5'-AAA ^{3'} | 5'-TTg ^{3'} | 5'-CTC ^{3'} | 5'-ggC ^{3'} | 5'-CTC ^{3'} |
| A* | + | + | + | | | |
| B* | + | + | + | | | |
| C* | + | + | + | | | |
| DRB1 | | | | + | + | |
| DRB3 | | | | + | + | |
| DRB5 | | | | + | | |
| DQB1 | | | | | + | |
| DPB1 | | | | | | + |

¹The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and 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.

²The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and 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.

101.555-06 – including *Taq* pol., IFU-01
101.555-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **92V**

Lot-specific information

www.olerup-ssp.com

PRODUCT DESCRIPTION

HLA-B*73 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*73:01 to B*73:02 alleles.

PLATE LAYOUT

Each HLA-B*73 test consists of 4 PCR reactions in an 8 well cut PCR plate. Wells 5 to 8 are empty.

| | | | | | | | |
|---|---|---|----|-------|-------|-------|-------|
| 1 | 2 | 3 | NC | empty | empty | empty | empty |
|---|---|---|----|-------|-------|-------|-------|

The 8 well PCR plate is marked with 'B73' in silver/gray ink.

Well No. 1 is marked with the Lot No. '92V'.

Wells 1 to 3– HLA-B*73 high resolution primers.

Well 4 – Negative Control (NC).

A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.

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

Only HLA-B*73 alleles will be amplified by the HLA-B*73 typing kit, except that the B*27:75 allele will be amplified by primer mix 1. Thus, the interpretation of HLA-B*73 typings is only marginally influenced by other groups of HLA-B alleles or alleles of other HLA Class I loci.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*73, i.e. **B*73:01 to B*73:02**, recognized by the HLA Nomenclature Committee in April 2014^{1,2} will be amplified by the primers in the HLA-B*73 SSP kit.

The HLA-B*73 kit enables separation of the confirmed HLA-B*73 alleles as listed in the IMGT/HLA database. An HLA allele is listed as confirmed by IMGT/HLA if it has been sequenced by more than a single laboratory or from multiple sources. Current allele confirmation status for HLA-B*73 alleles is listed below.

The HLA-B*73 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles

101.555-06 – including *Taq* pol., IFU-01
101.555-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **92V**

Lot-specific information

www.olerup-ssp.com

¹HLA-B alleles listed on the IMGT/HLA web page 2014-April-14, release 3.16.0, www.ebi.ac.uk/imgt/hla.

²Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page <http://hla.alleles.org/alleles/deleted.html>.

ALLELE CONFIRMATION STATUS

| Allele | Status ¹ |
|----------------|---------------------|
| B*73:01 | Confirmed |
| B*73:02 | Unconfirmed |

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2014-April-14, release 3.16.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-B*73 homo- and heterozygotes is available upon request.

101.555-06 – including *Taq* pol., IFU-01
101.555-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **92V**

Lot-specific information

www.olerup-ssp.com

SPECIFICITY TABLE

HLA-B*73 SSP subtyping

Specificities and sizes of the PCR products of the 3+1 primer mixes used for HLA-B*73 SSP subtyping

| Primer Mix | Size of spec. PCR product ¹ | Size of control band ² | Amplified HLA-B*73 alleles | Other amplified HLA-B alleles ³ |
|----------------|--|-----------------------------------|----------------------------|--|
| 1 | 150 bp | 800 bp | *73:01-73:02 | *27:75 |
| 2 | 220 bp | 1070 bp | *73:01 | |
| 3 | 220 bp | 1070 bp | *73:02 | |
| 4 ⁴ | | | Negative Control | |

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*73 SSP typings.

When the primers in a primer mix can give rise to HLA-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits, the alleles listed are specified according to amplicon length.

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 internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. 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 the B*27:75 allele will be amplified by primer mix 1.

⁴Primer mix 4 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by control primer pairs. PCR product sizes range from 75 to 200 base pairs. The PCR product generated by the control primer pair is 430 base pairs.

101.555-06 – including *Taq* pol., IFU-01
101.555-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **92V**

Lot-specific information

www.olerup-ssp.com

PRIMER SPECIFICATION

| Well No. | 1 | 2 | 3 |
|---------------------------|------------|------------|------------|
| Length of spec. | 150 | 220 | 220 |
| PCR product | | | |
| Length of int. | 800 | 1070 | 1070 |
| pos. control ¹ | | | |
| 5'-primer(s) ² | 165 | 379 | 379 |
| | 5' -ACC 3' | 5' -ACA 3' | 5' -ACA 3' |
| 3'-primer(s) ³ | 272 | 556 | 556 |
| | 5' -TgC 3' | 5' -gCC 3' | 5' -gCT 3' |
| Well No. | 1 | 2 | 3 |

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 1070 or 800 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the shorter, 800 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

²The nucleotide position 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 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.

101.555-06 – including *Taq* pol., IFU-01
101.555-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **92V**

Lot-specific information

www.olerup-ssp.com

| CELL LINE VALIDATION SHEET | | | | | | | | |
|--|------|------------|-----------|--------|-------------|-----------|-----------|-----------|
| HLA-B*73 SSP primer set² | | | | | | | | |
| | | | | | Well | | | |
| | | | | | 1 | 2 | 3 | |
| | | | | | Prod. No.: | 201077001 | 201077002 | 201077003 |
| IHWC cell line¹ | | | B* | | | | | |
| 1 | 9001 | SA | *07:02 | | - | - | - | |
| 2 | 9280 | LK707 | *52:01 | *73:01 | + | + | - | |
| 3 | 9011 | E4181324 | *52:01 | | - | - | - | |
| 4 | 9275 | GU373 | *15:10 | *53:01 | - | - | - | |
| 5 | 9009 | KAS011 | *37:01 | | - | - | - | |
| 6 | 9353 | SM | *39:01 | *51:01 | - | - | - | |
| 7 | 9020 | QBL | *18:01 | | - | - | - | |
| 8 | 9025 | DEU | *35:01 | | - | - | - | |
| 9 | 9026 | YAR | *38:01 | | - | - | - | |
| 10 | 9107 | LKT3 | *54:01 | | - | - | - | |
| 11 | 9051 | PITOUT | *44:03 | | - | - | - | |
| 12 | 9052 | DBB | *57:01 | | - | - | - | |
| 13 | 9004 | JESTHOM | *27:05 | | - | - | - | |
| 14 | 9071 | OLGA | *15:01 | *15:20 | - | - | - | |
| 15 | 9075 | DKB | *40:01 | | - | - | - | |
| 16 | 9037 | SWEIG007 | *40:02 | | - | - | - | |
| 17 | 9282 | CTM3953540 | *08:01 | *55:01 | - | - | - | |
| 18 | 9257 | 32367 | *14:01 | *56:01 | - | - | - | |
| 19 | 9038 | BM16 | *18:01 | | - | - | - | |
| 20 | 9059 | SLE005 | *40:01 | | - | - | - | |
| 21 | 9064 | AMALA | *15:01 | | - | - | - | |
| 22 | 9056 | KOSE | *35:03 | | - | - | - | |
| 23 | 9124 | IHL | *40:02 | *56:02 | - | - | - | |
| 24 | 9035 | JBUSH | *38:01 | | - | - | - | |
| 25 | 9049 | IBW9 | *14:02 | | - | - | - | |
| 26 | 9285 | WT49 | *58:01 | | - | - | - | |
| 27 | 9191 | CH1007 | *07:05 | *51:01 | - | - | - | |
| 28 | 9320 | BEL5GB | *44:02 | *44:03 | - | - | - | |
| 29 | 9050 | MOU | *44:03 | | - | - | - | |
| 30 | 9021 | RSH | *42:01 | | - | - | - | |
| 31 | 9019 | DUCAF | *18:01 | | - | - | - | |
| 32 | 9297 | HAG | *41:02 | | - | - | - | |
| 33 | 9098 | MT14B | *40:01 | | - | - | - | |
| 34 | 9104 | DHIF | *38:01 | | - | - | - | |
| 35 | 9302 | SSTO | *44:02 | | - | - | - | |
| 36 | 9024 | KT17 | *15:01 | *35:01 | - | - | - | |
| 37 | 9065 | HHKB | *07:02 | | - | - | - | |
| 38 | 9099 | LZL | *15:01 | | - | - | - | |
| 39 | 9315 | CML | *08:01 | *27:05 | - | - | - | |
| 40 | 9134 | WHONP199 | *13:02 | *46:01 | - | - | - | |
| 41 | 9055 | H0301 | *14:02 | | - | - | - | |
| 42 | 9066 | TAB089 | *46:01 | | - | - | - | |
| 43 | 9076 | T7526 | *46:01 | | - | - | - | |
| 44 | 9057 | TEM | *38:01 | | - | - | - | |
| 45 | 9239 | SHJO | *42:01 | *50:01 | - | - | - | |
| 46 | 9013 | SCHU | *07:02 | | - | - | - | |
| 47 | 9045 | TUBO | *51:01 | | - | - | - | |
| 48 | 9303 | TER-ND | *35:01 | *44:03 | - | - | - | |

¹The provided cell line HLA specificities are retrieved from the <http://www.ihwg.org/hla> web site. The specificity of an individual cell line may thus be subject to change.

101.555-06 – including *Taq* pol., IFU-01
101.555-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **92V**

Lot-specific information

www.olerup-ssp.com

²The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs.

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

101.555-06 – including *Taq* pol., IFU-01
101.555-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **92V**

Lot-specific information

www.olerup-ssp.com

101.555-06 – including *Taq* pol., IFU-01
101.555-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **92V**

Lot-specific information

www.olerup-ssp.com

101.555-06 – including *Taq* pol., IFU-01
101.555-06u – without *Taq* polymerase, IFU-02

Visit www.olerup-ssp.com for
“Instructions for Use” (IFU)

Lot No.: **92V**

Lot-specific information

www.olerup-ssp.com

ADDRESSES:

Manufacturer:

Olerup SSP AB, Franzengatan 5, SE-112 51 Stockholm, Sweden.

Tel: +46-8-717 88 27

Fax: +46-8-717 88 18

E-mail: info-ssp@olerup.com

Web page: <http://www.olerup-ssp.com>

Distributed by:

Olerup GmbH, Löwengasse 47 / 6, AT-1030 Vienna, Austria.

Tel: +43-1-710 15 00

Fax: +43-1-710 15 00 10

E-mail: support-at@olerup.com

Web page: <http://www.olerup.com>

Olerup Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382

Tel: 1-877-OLERUP1

Fax: 610-344-7989

E-mail: info.us@olerup.com

Web page: <http://www.olerup.com>

For information on *Olerup* SSP distributors worldwide, contact **Olerup GmbH**.