**101.532-48– including *Taq* polymerase,** IFU-01
**101.532-48u – without *Taq* polymerase,** IFU-02

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**Lot No.: 16V**

**Lot-specific information**

# *Olerup* SSP® HLA-B*27 – bulk

| | |
|---|---|
| **Product number:** | **101.532-48 – including *Taq* polymerase**<br>**101.532-48u – without *Taq* polymerase** |
| **Lot number:** | **16V** |
| **Expiry date:** | **2016-January-01** |
| **Number of tests:** | **48** |
| **Number of wells per test:** | **2** |
| **Storage - primer vials:** | **dark at -20°C** |
| **- PCR Master Mix:** | **-20°C** |
| **- Control DNAs:** | **-20°C** |
| **- Product Insert** | **RT** |

## This Product Description is only valid for Lot No. 16V.

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*27 LOT (30S)

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

The HLA-B*27 specificity and interpretation tables has been updated for the HLA-B alleles described since the previous *Olerup* SSP® HLA-B*27 lot **(Lot No. 30S)** was made.

The HLA-B*27 bulk primer set is unchanged compared to the previous lot.

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.

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# PRODUCT DESCRIPTION

## HLA-B*27 SSP typing

### CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-B27 specificity, B*27:01 to B*27:107.
The primer solutions consist of specific primer mixes, i.e. group-specific primers as well as a ***control primer pair*** matching non-allelic sequences.
Positive and negative control DNAs are included in the kit.
DNA 1; a B*27-positive DNA as a positive control, **IHW 9315, CML, B*08:01,27:05.**
DNA 2; a B*73-positive DNA as a negative control, **IHW 9280, LK707, B*52:01:01,73:01**. (A B*73:01-positive DNA was chosen as negative control, as this is most similar to the B*27 group of alleles in the primer matching regions.)
***PCR Master Mix complete with Taq,*** *Taq* polymerase, nucleotides, buffer, glycerol and cresol red, is included in the kit including *Taq* polymerase.

### INTERPRETATION

Due to the sharing of sequence motifs between HLA-B alleles, a few non-HLA-B*27 alleles will be amplified by primer mixes 1 and 2.
For further details see Specificity Table.

### UNIQUELY IDENTIFIED ALLELES

All the HLA-B*27 alleles, i.e. **B*27:01 to B*27:107**, recognized by the HLA Nomenclature Committee in October 2013[1,2] are identified by the primers in the HLA-B*27 SSP kit.

[1]HLA-B alleles listed on the IMGT/HLA web page 2013-October-11, release 3.14.0, www.ebi.ac.uk/imgt/hla.
[2]Alleles 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.

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# PROTOCOL

## DNA EXTRACTION

Extracted, highly pure DNA is needed for SSP typings. DNA samples to be used for PCR-SSP HLA typing should be re-suspended in $dH_2O$. The A260/A280 ratio should be 1.6 – 2.0 by UV spectrophotometry for optimal band visualization during electrophoresis.

We recommend automated DNA extraction with the QIAGEN EZ1 DSP DNA Blood System. ACD blood should be used as starting material.

Alternatively, the DNA can be extracted by any preferred method yielding pure DNA. When using alternative methods, the DNA concentration should be adjusted to 30 ng/µl. ***Do not use heparinised blood with these methods.***

| Recommended DNA concentration using: |
|---|
| EZ1-extracted DNA, 15 ng/µl. |
| DNA extracted by other methods, 30 ng/µl. |

Concentrations exceeding 50 ng/µl will increase the risk for nonspecific amplifications and weak extra bands, especially for HLA Class I high resolution SSP typings. If necessary, dilute the extracted DNA in $dH_2O$.

## PCR AMPLIFICATION

### *101.532-48 – including Taq polymerase*

For one HLA-B*27 bulk typing, dispense 5 µl of each of the 2 HLA-B*27 bulk primer solutions into an 8 tube strip of 0.2 ml PCR tubes; primer solution 1 into well 1, primer solution 2 into well 2.
For one HLA-B*27 typing, add at room temperature in a 0.5 ml tube:

4 x 2 µl = 8 µl DNA (30 ng/µl)
4 x 3 µl = 12 µl PCR Master Mix complete with *Taq* – mix well before taking your aliquot

Mix well, dispense 5 µl of the DNA-PCR Master Mix mixture into each of the 2 wells of an HLA-B*27 typing. Close the 8 tube PCR strip with an 8 strip lid.

Use a 96 well thermal cycler with a heated lid. The temperature gradient across the heating block should be ≤0.75°C.

***101.532-48u – without Taq polymerase***

For one HLA-B*27 bulk typing, dispense 5 µl of each of the 2 HLA-B*27 bulk primer solutions into an 8 tube strip of 0.2 ml PCR tubes; primer solution 1 into well 1, primer solution 2 into well 2.

For one HLA-B*27 typing, add at room temperature in a 0.5 ml tube:

4 x 2 µl = 8 µl DNA (30 ng/µl)
4 x 3 µl = 12 µl Master Mix without *Taq* – mix well before taking your aliquot
0.3 µl *Taq* polymerase (5 units/µl)

Mix well, dispense 5 µl of the DNA-PCR Master Mix mixture into each of the 2 wells of an HLA-B*27 typing. Close the 8 tube PCR strip with an 8 strip lid.

Use a 96 well thermal cycler with a heated lid. The temperature gradient across the heating block should be ≤0.75°C.

**PCR cycling parameters:**

| | | | |
|---|---|---|---|
| 1. 1 cycle | 94°C | 2 min | denaturation |
| 2. 10 cycles | 94°C | 10 sec. | denaturation |
| | 65°C | 60 sec. | annealing and extension |
| 3. 20 cycles | 94°C | 10 sec. | denaturation |
| | 61°C | 50 sec. | annealing |
| | 72°C | 30 sec. | extension |
| 4. End - hold | RT | | if less than 8 hours |
| | 4°C | | if longer than 8 hours |

Total reaction volume in each well, 10 µl.

The same PCR cycling parameters are used for all the *Olerup* SSP kits.

## AGAROSE GEL ELECTROPHORESIS

Prepare a 2% (w/v) agarose gel in 0.5 x TBE buffer. Dissolve the agarose by boiling in a microwave oven. Let the gel solution cool to 60°C. Stain the gel prior to casting with ethidium bromide (10 mg/ml), 5 µl per 100 ml gel solution. For maximal ease of handling use our ethidium bromide dropper bottles (Product No. 103.301-10), 1 drop of ethidium bromide solution per 50-75 ml of gel, or our GelRed™ dropper bottle (Product No. 103.302-05) 4 drops per 100-120 ml of gel solution. **Note: Ethidium bromide is a powerful carcinogen.**

Load the PCR products, preferably using an 8-channel pipette. Load a DNA size marker (100 base pair ladder, DNA Size Marker Product No. 103.202-100 or DNA Size Marker for short gel runs 103.203-100) in one well per row.

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Run the gel in 0.5 x TBE buffer, without re-circulation of the buffer, for 15-20 minutes at 8-10 V/cm.

## DOCUMENTATION AND INTERPRETATION

Put the gel on a UV transilluminator and document by photography.
Record the presence and absence of specific PCR products. The length of the specific PCR product is helpful in the interpretation of the results.
Record the presence of the internal positive control bands.
Lanes without either control band or specific PCR products should be repeated.

Interpret the typings with the ***lot-specific Interpretation and Specificity Tables***.

## PCR MASTER MIXES

### *101.532-48 – including Taq polymerase*

The PCR Master Mix complete with *Taq* contains:

| | |
|---|---|
| *Taq* polymerase | 0.4 unit per 10 µl SSP reaction |
| nucleotides | final concentration of each dNTP is 200 µM |
| PCR buffer | final concentrations: 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin |
| glycerol | final concentration of glycerol is 5% |
| cresol red | final concentration of cresol red is 100 µg/ml |

The same PCR Master Mix is used for all *Olerup* SSP kits including *Taq* polymerase.

The PCR Master Mix complete with *Taq* can be shipped at ambient temperature.
When stored at –20°C, the PCR Master Mix including *Taq* polymerase is stable for 33 months from the date of manufacture.

### *101.532-48u – without Taq polymerase*

The PCR Master Mix without *Taq* contains:

| | |
|---|---|
| nucleotides | final concentration of each dNTP is 200 µM |
| PCR buffer | final concentrations: 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 0.001% w/v gelatin |
| glycerol | final concentration of glycerol is 5% |
| cresol red | final concentration of cresol red is 100 µg/ml |

The same PCR Master Mix is used for all *Olerup* SSP kits without *Taq* polymerase.

The PCR Master Mix without *Taq* can be shipped at ambient temperature.
When stored at –20°C, the PCR Master Mix without *Taq* polymerase is stable for 33 months from the date of manufacture.

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# SPECIFICITY TABLE

## HLA-B*27 SSP typing

### Specificity and size of the PCR product of the two primer mixes used for HLA-B*27 SSP typing.

| Primer Mix | Size of spec. PCR product[1] | Size of control band[2] | Amplified HLA-B*27 alleles | Other amplified HLA-B alleles[3] |
|---|---|---|---|---|
| **1** | 145 bp | **430 bp** | *27:01-27:05:08, 27:05:10-27:05:22, 27:05:24-27:11, 27:13-27:15, 27:17, 27:19-27:21, 27:24-27:25, 27:27-27:28, 27:30, 27:32-27:74, 27:76, 27:78-27:84, 27:86-27:91, 27:93-27:100, 27:102-27:107 | *44:97 |
| **2**[4] | 95 bp | 515 bp | *27:01-27:05:15, 27:05:17-27:05:26, 27:08, 27:10, 27:12-27:13, 27:15-27:18, 27:23, 27:25-27:26, 27:28-27:29, 27:31, 27:36-27:40, 27:42, 27:44-27:45, 27:47-27:69, 27:71-27:75, 27:77, 27:79-27:80, 27:82-27:90:02, 27:92-27:101, 27:103-27:105 | *07:197, 37:02, 47:04-47:05, 48:26 |

[1]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*27 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 inherit 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

[2]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 430 base pairs or a band of 515 base pairs.
Well number 1 contains the primer pair giving rise to the shorter, 430 bp, internal positive control band in order to help in the correct orientation of the HLA-B*27 typing.
In the presence of a specific amplification the intensity of the control band often decreases.

[3]Due to the sharing of sequence motifs between HLA-B alleles a few non-HLA-B*27 alleles will be amplified by both primer mixes.

[4]HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

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# PRIMER SPECIFICATION

| **Well No.** | **1** | **2** |
|---|---|---|
| **Length of spec. PCR product** | **145** | **95** |
| | | |
| **Length of int. pos. control[1]** | **430** | **515** |
| | | |
| **5'-primer[2]** | **167** | **363** |
| | **5' -gCT 3'** | **5' -AAT 3'** |
| | | |
| **3'-primer[3]** | **272** | **418** |
| | **5' -TgC 3'** | **5' -gTC 3'** |
| **Well No.** | **1** | **2** |

[1]The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table.
Well number 1 contains the shorter, 430 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.

[2]The 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.

[3]The 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.

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# CELL LINE VALIDATION SHEET

## HLA-B*27 bulk SSP kit[2]

| | | | | | Well | |
|---|---|---|---|---|---|---|
| | | | | | 1 | 2 |
| | | | | | Production No. | 201328301 | 201328302 |
| | IHWC cell line[1] | | HLA-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 | - | - |

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[1]The 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.

[2]The 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.

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