**101.331.24/06 – including *Taq* pol.,** IFU-01
**101.331.24u/06u – without *Taq* pol.,** IFU-02

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**Lot No.: 40X**

**Lot-specific information**

# *Olerup* SSP® DPA1

| | |
|---|---|
| **Product number:** | **101.331-24/06 – including *Taq* pol.**<br>**101.331-24u/06u – without *Taq* pol.** |
| **Lot number:** | **40X** |
| **Expiry date:** | **2017-April-01** |
| **Number of tests:** | **24 test – Product No. 101.331-24/24u**<br>**6 tests – Product No. 101.331-06/06u** |
| **Number of wells per test:** | **15+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. 40X.

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

## CHANGES COMPARED TO THE PREVIOUS *OLERUP* SSP® DPA1 LOT (71S)

A well containing Negative Control primer pairs has been added.

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

The DPA1 primer set, specificity and interpretation tables have been updated for the DPA1 alleles described since the previous *Olerup* SSP® DPA1 lot was made **(Lot No. 71S)**. The kit design is based on IMGT/HLA database 3.17.0.

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 added, exchanged or modified.

| Well | 5'-primer | 3'-primer | rationale |
|---|---|---|---|
| 11 | Added | - | 5'-primer added from well 13. |
| 13 | Moved, added | Added | 5'-primers moved to wells 11 and 14, primer pair added from well 16. |
| 14 | Added | - | 5'-primer added from well 13. |
| 16 | Moved | Moved | Primer pair moved to well 13, Negative Control. |

**Lot No.: 40X** **Lot-specific information**

Well **16** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup* SSP® HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

HLA-specific PCR product sizes range from 75 to 200 base pairs.
The PCR product generated by the positive control primer pair is 430 base pairs.

| **Length of PCR product** | **105** | **200** | **105** | **80** | **75** | **80** | **85** |
|---|---|---|---|---|---|---|---|
| **5'-primer[1]** | **164** | **340** | **440** | **45** | **45** | **43** | **36** |
| | **5'-CAC3'** | **5'-Agg3'** | **5'-TTA3'** | **5'-Tgg3'** | **5'-Tgg3'** | **5'-Tgg3'** | **5'-TAC3'** |
| | | | | | | | **36** |
| | | | | | | | **5'-TAT3'** |
| **3'-primer[2]** | **231** | **2nd I** | **507** | **59** | **58** | **57** | **47** |
| | **5'-TgC3'** | **5'-AAA3'** | **5'-TTg3'** | **5'-CTC3'** | **5'-ggC3'** | **5'-CTC3'** | **5'-ACA3'** |
| | | | | | | | **48** |
| | | | | | | | **5'-gCA3'** |
| | | | | | | | **48** |
| | | | | | | | **5'-gCC3'** |
| | | | | | | | **52** |
| | | | | | | | **5'-TgT3'** |
| **A*** | **+** | **+** | **+** | | | | |
| **B*** | **+** | **+** | **+** | | | | |
| **C*** | **+** | **+** | **+** | | | | |
| **DRB1** | | | | **+** | **+** | | |
| **DRB3** | | | | **+** | **+** | | |
| **DRB5** | | | | **+** | | | |
| **DQB1** | | | | | **+** | | |
| **DPB1** | | | | | | **+** | |
| **DQA1** | | | | | | | **+** |

[1]The 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 codonnumbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.
[2]The 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.

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**Lot No.: 40X** **Lot-specific information**

# PRODUCT DESCRIPTION

## DPA1 SSP subtyping

### CONTENT

The primer set contains 5'- and 3'-primers for identifying the DPA1*01:03 to DPA1*04:01 alleles.

### PLATE LAYOUT

Each test consists of 16 PCR reactions in a 16 well cut PCR plate.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |

The 16 well cut PCR plate is marked with 'DPA1' in silver/gray ink.
Well No. 1 is marked with the Lot No. '40X'.
Wells 1 to 15 – DPA1 high resolution primers.
Well 16 – 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 16 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 DPA1 alleles will be amplified by the DPA1 typing kit. Thus, the interpretation of DPA1 typings is not influenced by the DPA2 gene.
For further details see Specificity Table.

### UNIQUELY IDENTIFIED ALLELES

All the phenotypically different DPA1 alleles, i.e. **DPA1*01:03 to DPA1*01:12, DPA1*02:01 to DPA1*02:05, DPA1*03:01 to DPA1*03:03 and DPA1*04:01**, recognized by the HLA Nomenclature Committee in July 2014[1,2] will give rise to unique amplification patterns by the primers in the DPA1 typing kit.

The DPA1 typing kit cannot distinguish the following silent mutations: DPA1*01:03:01:01-DPA1*01:03:02 and DPA1*01:03:04, the DPA1*01:06:01-DPA1*01:06:02, the DPA1*02:01:01-DPA1*02:01:07 and the DPA1*02:02:01 to DPA1*02:02:06 alleles.

[1]DPA1 alleles listed on the IMGT/HLA web page 2014-July-25, release 3.17.0, 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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## RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DPA1 homo- and heterozygotes is available upon request.

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**Lot No.: 40X** **Lot-specific information**

# SPECIFICITY TABLE

## DPA1 SSP typing

### Specificities and sizes of the PCR products of the 15+1 primer mixes used for DPA1 SSP typing

| Primer Mix | Size of spec. PCR product[1] | Size of control band[2] | Amplified DPA1[3] alleles |
|---|---|---|---|
| **1**[4] | 85 bp | **515 bp** | *01:03:01:01-01:03:02, 01:03:04-01:05, 01:07-01:12, 04:01 |
| **2** | 255 bp | **515 bp** | *01:03:01:01-01:04, 01:06:01-01:12 |
| **3** | 205 bp | 430 bp | *01:03:01:01-01:03:04, 01:06:01-01:07, 01:09-01:12, 03:01-03:02 |
| **4**[4,5] | 115 bp | 430 bp | *01:04, 01:08, 03:03 |
| **5**[4] | 105 bp | 430 bp | *01:05, 02:01:01-02:05, 04:01 |
| **6**[8] | 160 bp<br>195 bp | **515 bp** | *01:10, 02:04<br>*01:06:01-01:06:02 |
| **7**[4] | 100 bp | 430 bp | *01:06:01-01:06:02, 02:01:01-02:01:07 |
| **8**[4] | 100 bp | 430 bp | *02:02:01-02:02:06, 02:04-02:05 |
| | | | |
| **9** | 205 bp | 430 bp | *02:02:01-02:02:06, 02:04-02:05, 03:02 |
| **10**[4] | 85 bp | **515 bp** | *01:03:03, 02:03, 03:01-03:03 |
| **11**[4,6,7] | 90 bp<br>135 bp | **515 bp** | *01:12, 03:01, 03:03<br>*01:07 |
| **12** | 205 bp | 430 bp | *04:01 |
| **13**[4] | 100 bp | 430 bp | *01:09 |
| **14** | 130 bp | **515 bp** | *01:07-01:08, 01:11, 02:05 |
| **15**[7] | 245 bp | 430 bp | *01:03:03, 03:01-03:03 |
| **16**[9] | | | **Negative Control** |

[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 DPA1 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.

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**Lot No.: 40X** **Lot-specific information**

[2]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 longer, 515 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.

[3]For several DPA1 alleles 1st and/or 3rd exon(s) and beyond, as well as intron nucleotide sequences, are not available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

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

[5]Primer mix 4 may faintly amplify the DPA1*04:01 allele.

[6]Primer mix 11 may have tendencies of unspecific amplifications.

[7]Primer mixes 11 and 15 may give rise to a lower yield of HLA-specific PCR product than the other DPA1 primer mixes.

[8]Primer mix 6 may give rise to a lower yield of HLA-specific PCR product than the other DPA1 primer mixes in the DPA1*01:06:01-01:06:02 and 02:04 alleles.

[9]Primer mix 16 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by the control primer pairs matching the human growth hormone gene. HLA-specific PCR product sizes range from 75 to 200 base pairs and the PCR product generated by the HGH positive control primer pair is 430 base pairs.

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# PRIMER SPECIFICATION

| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Length of spec. | 85 | 255 | 205 | 115 | 105 | 160 | 100 | 100 | 205 | 85 | 90 | 205 |
| PCR product | | | | | | 195 | | | | | 135 | |
| Length of int. pos. control[1] | 515 | 515 | 430 | 430 | 430 | 515 | 430 | 430 | 430 | 515 | 515 | 430 |
| 5'-primer(s)[2] | 15(138) | 11(125) | 28(177) | 3(103) | 84(345) | 31(185) | 11(125) | 11(125) | 11(125) | 15(138) | 50(244) | 17(145) |
| | 5' -ACg 3' | 5' -CgC 3' | 5' -gAA 3' | 5' -Cgg 3' | 5' -AAT 3' | 5' -gCA 3' | 5' -CgC 3' | 5' -CAT 3' | 5' -CAT 3' | 5' -ACC 3' | 5' -AAA 3' | 5' -gAA 3' |
| | | | | | | 43(222) | | | | | 66(290) | |
| | | | | | | 5' -TgT 3' | | | | | 5' -ATC 3' | |
| 3'-primer(s)[3] | 30(184) | 82(340) | 82(340) | 28(177) | 2nd I | 68(298) | 30(184) | 30(184) | 66(290) | 30(184) | 82(340) | 72(310) |
| | 5' -CAT 3' | 5' -ggT 3' | 5' -ggT 3' | 5' -TCg 3' | 5' -ggC 3' | 5' -gTC 3' | 5' -CTg 3' | 5' -CTg 3' | 5' -TCA 3' | 5' -CAT 3' | 5' -ggT 3' | 5' -AgC 3' |
| | | | | | | 82(340) | | | | | | |
| | | | | | | 5' -ggT 3' | | | | | | |
| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |

| Well No. | 13 | 14 | 15 |
|---|---|---|---|
| Length of spec. | 100 | 130 | 245 |
| PCR product | | | |
| Length of int. pos. control[1] | 430 | 515 | 430 |
| 5'-primer(s)[2] | 3(103) | 50(242) | 15(138) |
| | 5' -Cgg 3' | 5' -CCg 3' | 5' -ACC 3' |
| | | 50(244) | |
| | | 5' -AAA 3' | |
| 3'-primer(s)[3] | 23(161) | 76(320) | 82(340) |
| | 5' -ACg 3' | 5' -AAT 3' | 5' -ggT 3' |
| | | 82(340) | |
| | | 5' -ggT 3' | |
| Well No. | 13 | 14 | 15 |

[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 longer, 515 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

## DPA1 SSP kit[2]

| | | | | | Well | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| | | | | Lot No.: | 201323201 | 201323202 | 201323203 | 201323204 | 201323205 | 201323206 | 201323207 | 201323208 | 201323209 | 201323210 | 201442411 | 201323212 | 201323216 | 201442414 | 201323215 |
| | IHWC cell line[1] | | DPA1 | | | | | | | | | | | | | | | | |
| 1 | 9001 | SA | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 9280 | LK707 | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 9011 | E4181324 | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 9275 | GU373 | *02:01 | *04:01 | + | - | - | - | + | - | + | - | - | - | - | + | - | - | - |
| 5 | 9009 | KAS011 | *01:03 | *02:01 | + | + | + | - | + | - | + | - | - | - | - | - | - | - | - |
| 6 | 9353 | SM | *02:02 | | - | - | - | - | + | - | - | + | + | - | - | - | - | - | - |
| 7 | 9020 | QBL | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 8 | 9025 | DEU | *01 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 9 | 9026 | YAR | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 10 | 9107 | LKT3 | *02:02 | | - | - | - | - | + | - | - | + | + | - | - | - | - | - | - |
| 11 | 9051 | PITOUT | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 12 | 9052 | DBB | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 13 | 9004 | JESTHOM | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 14 | 9071 | OLGA | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 15 | 9075 | DKB | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 16 | 9037 | SWEIG007 | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 17 | 9282 | CTM3953540 | *01:03 | *02:01 | + | + | + | - | + | - | + | - | - | - | - | - | - | - | - |
| 18 | 9257 | 32367 | *01:03 | *03:01 | + | + | + | - | - | - | - | - | - | + | + | - | - | - | + |
| 19 | 9038 | BM16 | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 20 | 9059 | SLE005 | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 21 | 9064 | AMALA | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 22 | 9056 | KOSE | *01:03 | *02:01 | + | + | + | - | + | - | + | - | - | - | - | - | - | - | - |
| 23 | 9124 | IHL | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 24 | 9035 | JBUSH | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 25 | 9049 | IBW9 | *02:01 | | - | - | - | - | + | - | + | - | - | - | - | - | - | - | - |
| 26 | 9285 | WT49 | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 27 | 9191 | CH1007 | *01:03 | *04:01 | + | + | + | - | + | - | - | - | - | - | - | + | - | - | - |
| 28 | 9320 | BEL5GB | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 29 | 9050 | MOU | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 30 | 9021 | RSH | *02:02 | *03:01 | - | - | + | - | + | - | - | + | + | + | + | - | - | - | + |
| 31 | 9019 | DUCAF | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 32 | 9297 | HAG | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 33 | 9098 | MT14B | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 34 | 9104 | DHIF | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 35 | 9302 | SSTO | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 36 | 9024 | KT17 | *02:02 | | - | - | - | - | + | - | - | + | + | - | - | - | - | - | - |
| 37 | 9065 | HHKB | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 38 | 9099 | LZL | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 39 | 9315 | CML | *01:03 | *02:01 | + | + | + | - | + | - | + | - | - | - | - | - | - | - | - |
| 40 | 9134 | WHONP199 | *02:02 | | - | - | - | - | + | - | - | + | + | - | - | - | - | - | - |
| 41 | 9055 | H0301 | *02:01 | | - | - | - | - | + | - | + | - | - | - | - | - | - | - | - |
| 42 | 9066 | TAB089 | *02:02 | | - | - | - | - | + | - | - | + | + | - | - | - | - | - | - |
| 43 | 9076 | T7526 | *04:01 | | + | - | - | - | + | - | - | - | - | - | - | + | - | - | - |
| 44 | 9057 | TEM | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 45 | 9239 | SHJO | *01:03 | *03:01 | + | + | + | - | - | - | - | - | - | + | + | - | - | - | + |
| 46 | 9013 | SCHU | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 47 | 9045 | TUBO | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 48 | 9303 | TER-ND | *01:03 | | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |

[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.

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**Lot No.: 40X** **Lot-specific information**

[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.
No DNAs carrying the allele to be amplified by primer solutions 6, 13 and 14 were available. The specificities of the primers in primer solutions 6 and 14 were tested by separately adding one additional 5'-primer and one additional 3'-primer, respectively. In primer solution 13 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solutions 6, 11 and 14 one 5'-primer was not possible to test, and in primer solutions 6 and 14 one 3'-primer was not possible to test.

**101.331.24/06 – including *Taq* pol.,** IFU-01
**101.331.24u/06u – without *Taq* pol.,** IFU-02

Visit www.olerup-ssp.com for **"Instructions for Use" (IFU)**

**Lot No.: 40X** **Lot-specific information**

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