Product Insert

101.564-06 – including *Taq* polymerase, IFU-01 101.564-06u – without *Taq* polymerase, IFU-02

Lot No.: **0M7**

Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

Lot-specific information Olerup SSP® HLA-B*45

| Product number: | 101.564-06 – including <i>Taq</i> polymerase 101.564-06u – without <i>Taq</i> polymerase |
|--|---|
| Lot number: | 0M7 |
| Expiry date: | 2025-03-01 |
| Number of tests: | 6 |
| 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. 0M7.

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*45 LOT (3H5).

- The product documentation has been updated for new alleles of IMGT 3.42.0.
- The kit resolution focuses on common and well documented (CWD) alleles¹.
- ¹As described in section Uniquely Identified Alleles.

The HLA-B*45 specificity and interpretation tables have been updated for the HLA-B alleles described since the previous *Olerup* SSP[®] HLA-B*45 lot was made (Lot No. 3H5).

The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot (Lot No. 3H5).

| Well | 5'-primer | 3'-primer | rationale |
|------|-----------|------------|--|
| 2 | - | Exchanged, | 3'-primer exchanged for the B*45:01:09 allele. |
| | | removed | 3'-primer removed. |

¹S. J. Mack, P. Cano, J. A. Hollenbach et al. Common and well-documented HLA alleles: 2012 update to the CWD catalogue. Tissue Antigens, 2013, 81, 194–203 101.564-06 – including *Taq* polymerase, IFU-01 101.564-06u – without *Taq* polymerase, IFU-02 Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

Lot No.: **0M7**

Lot-specific information

Well **16** contains <u>Negative Control primer pairs</u>, that will amplify the majority 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 200 base pairs.

| Length of PCR | 105 | 200 | 105 | 80 | 75 | 80 | 85 |
|------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| product | | | | | | | |
| 5'-primer ¹ | 164 | 340 | 440 | 45 | 45 | 43 | 36 |
| | 5'-CAC3' | ^{5'} -Agg ^{3'} | ^{5'} -TTA3' | ^{5′} -Tgg³′ | ^{5'} -Tgg ^{3'} | ^{5'} -Tgg ^{3'} | ^{5'} -TAC ^{3'} |
| | | | | | | | 36 |
| | | | | | | | ^{5'} -TAT ^{3'} |
| 3'-primer ² | 231 | 2 nd I | 507 | 59 | 58 | 57 | 47 |
| | ^{5'} -TgC ^{3'} | ⁵ '-AAA ^{3'} | ^{5'} -TTg ^{3'} | ^{5'} -CTC ^{3'} | ^{5'} -ggC ^{3'} | ^{5'} -CTC ^{3'} | ^{5'} -ACA ^{3'} |
| | | | | | | | 48 |
| | | | | | | | ^{5'} -gCA ^{3'} |
| | | | | | | | 48 |
| | | | | | | | ^{5'} -gCC ^{3'} |
| | | | | | | | 52 |
| | | | | | | | ^{5′} -TgT ^{3′} |
| A* | + | + | + | | | | |
| B* | + | + | + | | | | |
| C* | + | ÷ | + | | | | |
| DRB1 | | | | + | + | | |
| DRB3 | | | | + | + | | |
| DRB5 | | | | ÷ | | | |
| DQB1 | | | | | + | | |
| DPB1 | | | | | | + | |
| DQA1 | | | | | | | + |

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 codonnumbering as on the <u>www.ebi.ac.uk/imgt/hla</u> 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 <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given.

Product Insert

101.564-06 – including *Taq* polymerase, IFU-01 101.564-06u – without *Taq* polymerase, IFU-02 Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

Lot No.: **0M7**

Lot-specific information PRODUCT DESCRIPTION

HLA-B*45 SSP typing

CONTENT

The primer set contains 5'- and 3'-primers for identifying the B*45:01 to B*45:27 alleles.

PLATE LAYOUT

Each HLA-B*45 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 | NC |

The 16 well PCR plate is marked with 'HLA-B*45' in silver/gray ink.

Well No. 1 is marked with the Lot No. '0M7'.

Wells 1 to 15 – HLA-B*45 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

Due to the sharing of sequence motifs between HLA-B alleles non-HLA-B*45 alleles will be amplified by some primer mixes. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the HLA-B*45, i.e. **B*45:01 to B*45:27**, recognized by the HLA Nomenclature Committee in October $2020^{1,2}$ will be amplified by the primers in the HLA-B*45 SSP kit³.

The HLA-B*45 kit enables separation of the confirmed HLA-B*45 alleles as listed in the IMGT/HLA database 3.23.0. 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*45 alleles is listed below.

The HLA-B*45 kit also enables identification of many null and alternatively expressed alleles.

¹HLA-B alleles listed on the IMGT/HLA web page 2020-October-15, release 3.42.0, <u>www.ebi.ac.uk/imgt/hla</u>.

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 <u>http://hla.alleles.org/alleles/deleted.html</u>.



Product Insert

101.564-06 – including *Taq* polymerase, IFU-01 101.564-06u – without *Taq* polymerase, IFU-02

Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

Lot No.: 0M7

Lot-specific information

³The HLA-B*45 primer set cannot separate the following alleles. These alleles can be distinguished by the HLA-B low resolution kit and/or the HLA-B*44 high resolution kit.

Alleles

B*45:22, B*44:15:01:01-44:15:01:02, 44:18

ALLELE CONFIRMATION STATUS

| Allele | Status ¹ | Allele | Status ¹ |
|------------|---------------------|---------|---------------------|
| B*45:01:01 | Confirmed | B*45:10 | Confirmed |
| B*45:01:02 | Unconfirmed | B*45:11 | Unconfirmed |
| B*45:02 | Confirmed | B*45:12 | Unconfirmed |
| B*45:03 | Unconfirmed | B*45:13 | Unconfirmed |
| B*45:04 | Unconfirmed | B*45:14 | Confirmed |
| B*45:05 | Unconfirmed | B*45:15 | Unconfirmed |
| B*45:06 | Unconfirmed | B*45:16 | Unconfirmed |
| B*45:07 | Unconfirmed | B*45:17 | Unconfirmed |
| B*45:08 | Unconfirmed | B*45:18 | Unconfirmed |
| B*45:09 | Unconfirmed | | |

¹Allele status "confirmed" or "unconfirmed" as listed on the IMGT/HLA web page 2016-January-19, release 3.23.0, <u>www.ebi.ac.uk/imgt/hla</u>.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-B*45 homo- and heterozygotes is available upon request.



101.564-06 – including *Taq* polymerase, IFU-01 101.564-06u – without *Taq* polymerase, IFU-02 Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

Lot No.: **0M7**

Lot-specific information SPECIFICITY TABLE

HLA-B*45 SSP subtyping

Specificities and sizes of the PCR products of the 15+1 primer mixes used for HLA-B*45 SSP subtyping

| Primer Mix | Size of spec. PCR product ¹ | Size of control band ² | Amplified HLA- B*45 alleles ³ | Other amplified HLA-B alleles |
|---------------|--|---|---|---|
| 1 | 255 bp | 800 bp | *45:01:01:01- 45:03, 45:05- 45:07, 45:09, 45:11-45:23, 45:25, 45:27 | *44:15:01:01-44:15:01:02, 44:18, 44:20, 44:47, 44:100, 44:197, 44:339, 49:20, 50:02:01:01- 50:02:01:02, 50:56, 51:23, 51:108, 52:15, 56:74 |
| 2 | 150 bp | 1070 bp | *45:01:01:01- 45:21, 45:23- 45:25, 45:27 | *07:133, 08:155, 15:46, 15:53, 15:106, 15:143, 15:212, 15:525, 18:48, 18:107, 35:19, 35:47, 35:63, 35:154, 35:217, 35:274, 35:317, 35:450, 39:69, 40:01:01-40:11:02, 40:14:01-40:16:01:02, 40:18, 40:20:01:01-40:20:01:02, 40:22N-40:40, 40:42-40:45, 40:48-40:78, 40:80-40:92, 40:94- 40:95, 40:97-40:108, 40:111-40:116, 40:118N- 40:136, 40:138-40:156, 40:158-40:184, 40:186:01-40:187, 40:189, 40:191-40:200, 40:202-40:206, 40:210-40:245, 40:247-40:264, 40:266-40:280, 40:282-40:288, 40:290-40:291N, 40:293-40:307, 40:309-40:339, 40:341-40:349, 40:351-40:364, 40:366-40:393, 40:395-40:425, 40:427-40:429, 40:431-40:468, 41:01:01:01- 41:13, 41:15-41:45N, 41:47-41:59, 41:61-41:66, 44:09, 44:46:01-44:46:02, 44:75, 44:90, 44:129, 44:131, 44:254, 44:277, 47:02, 48:41, 50:01:01:01-50:02:01:02, 50:04-50:05, 50:07- 50:20, 50:31-50:72N, 56:67 |
| 3 | 160 bp | 800 bp | *45:01:01:01- 45:01:11, 45:04- 45:08, 45:10- 45:25, 45:27 | *44:15:01:01-44:15:01:02, 44:18, 44:140, 55:56, 56:13, 82:01:01:01-82:04 |
| 4 | 160 bp | 800 bp | *45:03, 45:07 | *35:485, 37:25 |
| 5 | 195 bp | 800 bp | *45:02 | *38:158, 44:117, 44:251, C*04:345, C*05:102, C*05:184, C*08:93, C*15:25 |
| 6 | 255 bp | 1070 bp | *45:04, 45:08, 45:10 | *07:78, 07:84, 08:09, 08:84, 08:254, 13:02:01:01- 13:04, 13:08-13:09, 13:14-13:16, 13:18-13:19, 13:27, 13:30-13:35, 13:37-13:38, 13:40-13:42, 13:44-13:49N, 13:53-13:56:02N, 13:58-13:59, 13:62, 13:65-13:70, 13:72, 13:74-13:75, 13:81- 13:82, 13:84-13:85, 13:88-13:89, 13:91, 13:93- 13:94, 13:96-13:105, 13:111-13:121, 13:123Q- 13:128, 13:130-13:134, 13:136-13:137N, 13:139N-13:140, 13:143, 13:145, 13:147, 14:37, 15:04:01:01-15:04:04, 15:16:01:01-15:16:04, 15:42, 15:67, 15:83:01:01-15:83:01:02, 15:95, 15:137, 15:155, 15:222, 15:254, 15:293, 15:303, 15:310, 15:362, 15:408, 15:485, 15:499, 15:517, 15:531, 15:550, 15:552, 27:14, 27:81, 27:130, |

Product Insert

101.564-06 – including *Taq* polymerase, IFU-01 101.564-06u – without *Taq* polymerase, IFU-02 Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

| Lo | t No.: 0M7 | | Lot-specific in | nformation |
|----|--------------------------|---------|-----------------|---|
| | <u>t No.: 0M7</u> | | Lot-specific in | 27:153, 35:37, 35:60, 35:235, 35:265, 35:273, 35:276, 39:06:01-39:06:06, 39:33-39:34, 39:50, 39:57, 39:62, 39:64, 39:83:01-39:83:03, 39:90, 39:102, 39:109, 39:127, 39:129, 39:132, 39:140- 39:141, 39:154, 40:06:01:01-40:06:12, 40:06:14- 40:06:25, 40:44, 40:53, 40:70:01-40:70:02, 40:75, 40:83, 40:86, 40:93, 40:95-40:96, 40:103, 40:109- 40:110, 40:127, 40:131, 40:148, 40:159, 40:161- 40:162, 40:165, 40:167, 40:177, 40:190, 40:230, 40:244, 40:256N, 40:268, 40:270, 40:275, 40:294- 40:295, 40:298:01-40:298:02, 40:306-40:307, 40:311, 40:313, 40:317-40:318, 40:330, 40:340- 40:342, 40:348-40:350, 40:354, 40:361N-40:364, 40:366, 40:373-40:374, 40:376, 40:387-40:389, 40:408, 40:411, 40:423, 40:425, 40:427, 40:434, 40:460, 40:464, 40:468, 41:01:01:01-41:01:07, 41:05-41:07, 41:09, 41:12, 41:14, 41:16-41:17, 41:20-41:22, 41:25-41:26, 41:28-41:30, 41:32- 41:35, 41:37, 41:44, 41:50, 41:53-41:54, 41:56Q- 41:57, 41:59-41:61, 41:64, 41:66, 42:04, 44:459, 46:11, 46:18, 46:32:01-46:32:02, 49:01:01:01- 49:14, 49:16-49:19N, 49:21-49:46, 49:48-49:60N, 49:62-49:69, 50:01:01:01-50:01:17, 50:04-50:111, 50:13-50:16, 50:18-50:20, 50:31-50:50, 50:52- 50:55, 50:57-50:72N, 51:01:01:01-51:22, 51:24:01- 51:26:05, 51:26-51:41N, 51:43-51:44N, 51:48- 51:55, 51:57-51:58, 51:60-51:61:02, 51:63:01- 51:63:02, 51:65-51:69, 51:71-51:20, 51:124.01- 51:24:05, 51:226, 51:228-51:240, 51:282- 51:107, 51:109-51:134, 51:136-51:138, 51:140- 51:147, 51:149N-51:174:02, 51:176-51:187, 51:189-51:193, 51:195-51:211, 51:224-51:291, 51:221-51:226, 51:228-51:280, 51:282- 51:107, 51:109, 51:311-51:318N, 51:320-51:324, 52:01:01:01-55:01:06, 55:01:00, 56:01:01:01-54:05N, 54:07-54:08N, 54:10-54:37, 54:39-54:43, 55:01:01:01-55:01:06, 55:01:08-55:03, 55:05, 55:07, 55:09-55:13, 55:15-55:48, 55:50, 55:22- 55:62, 55:64-55:85, 55:87-55:109, 56:11:01:01- 55:01:11, 56:01:16, 56:05:01-56:02, 56:64- 56:65, 56:67-56:73, 56:75, 56:01-56:02, 56:64- 56:65, 56:67-56:73, 56:75, 56:01-56:02, 56:64- 56:65, 56:67-56:73, 56:75, 56:01-56:02, 56:64- 56:65, 56:67-56:73, 56:75, 55:00, 55:22- 56: |
| - | 000 1 | | * 4 5 0 5 | 78:01:01:02-78:10, C*03:464, C*05:152, C*07:463, C*07:758, C*12:183, C*14:92 |
| 7 | 230 bp | 1070 bp | *45:05 | *07.122 40.166 44.00 |
| 84 | 105 bp | 1070 bp | *45:06 | *07:133, 40:166, 44:90 |
| 9 | 245 bp | 1070 bp | *45:08 | *08:09, 08:84, 08:254, 13:46, 13:53, 13:131, 14:37, 15:42, 15:83:01:01-15:83:01:02, 15:485, 35:60, 35:276, 39:06:01-39:06:06, 39:34, 39:57, 39:62, 39:64, 39:83:01-39:83:03, 39:90, 39:109, 39:127, 39:129, 39:132, 39:140-39:141, 39:154, |

Product Insert

101.564-06 – including *Taq* polymerase, IFU-01 101.564-06u – without *Taq* polymerase, IFU-02 Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

| | Lot No.: 0M7 | | Lot-specific inf | ormation |
|----------|---------------------|--------------------------|------------------|---|
| | | | | 41:01:01:01-41:01:07, 41:05-41:07, 41:09, 41:12, 41:14, 41:16, 41:20-41:22, 41:25-41:26, 41:28- 41:30, 41:32-41:35, 41:37, 41:44, 41:50, 41:53- 41:54, 41:57, 41:59-41:61, 41:64, 41:66, 42:04, 46:18, 50:45, 50:49, 51:21, 51:36, 51:101, 51:143, 51:153, 51:157, 51:197, 51:280, 51:295, 51:300, 52:37, 52:71, 52:84, 54:01:01:01-54:02, 54:04- 54:05N, 54:07-54:08N, 54:10-54:25, 54:27-54:32, 54:34-54:37, 54:39, 54:41-54:43, 55:01:01:01- 55:01:06, 55:01:08-55:03, 55:05, 55:07, 55:10- 55:13, 55:15-55:21, 55:23, 55:25-55:48, 55:50, 55:52-55:85, 55:87-55:109, 56:23, 56:54, 59:01:01:01-59:03, 59:05-59:08, 59:10N-59:11, 78:07, C*05:152, C*07:463, C*07:758, C*12:183, C*14:92 |
| 10 | 160 bp | 1070 bp | *45:09 | *08:28, 08:35, 08:37, 08:89, 08:107, 15:51, 15:179:01-15:179:02, 15:199, 15:218Q, 15:518, 18:56, 18:113, 35:115, 35:260, 37:35, 37:56, 41:24, 41:30, 41:51, 42:09, 44:17, 44:230, 44:308, 51:97, 53:22, 57:24, 57:119, C*07:68, C*07:140:01-07:140:02, C*07:151, C*07:302 |
| 11 | 245 bp | 1070 bp | *45:10 | *07:78, 07:84, 13:02:01:01-13:02:32, 13:08-13:09, 13:14-13:16, 13:18-13:19, 13:27, 13:30-13:35, 13:37-13:38, 13:40-13:42, 13:44-13:45, 13:47, 13:49N, 13:54-13:56:02N, 13:58-13:59, 13:65- 13:70, 13:72, 13:74-13:75, 13:81-13:82, 13:84- 13:85, 13:88-13:89, 13:91, 13:93-13:94, 13:96- 13:105, 13:111-13:119, 13:121, 13:123Q-13:128, 13:130, 13:132-13:134, 13:136-13:137N, 13:139N-13:140, 13:143, 13:145, 13:147, 27:14, 27:81, 27:130, 39:50, 40:06:01:01-40:06:25, 40:44, 40:53, 40:70:01-40:70:02, 40:75, 40:83, 40:86, 40:93, 40:95-40:96, 40:103, 40:109- 40:110, 40:127, 40:131, 40:148, 40:159, 40:161- 40:162, 40:165, 40:167, 40:177, 40:190, 40:230, 40:244, 40:256N, 40:268-40:270, 40:275, 40:294- 40:295, 40:298:01-40:298:02, 40:306-40:307, 40:311, 40:313, 40:317-40:318, 40:330, 40:340- 40:342, 40:348-40:350, 40:354, 40:361N-40:364, 40:366, 40:373-40:374, 40:376, 40:387-40:389, 40:408, 40:411, 40:423, 40:425, 40:427, 40:434, 40:445, 40:449, 40:451, 40:453-40:454, 40:459- 40:460, 40:464, 40:468, 49:07, 50:31, 51:10, 51:16, 51:31, 51:34, 51:82, 51:93, 52:08, 52:67, 54:26, 55:09, 55:22, 55:24, 59:09, 73:01:01:01- 73:03, 78:08 |
| 12 13 | 205 bp 300 bp | 1070 bp 800 bp | *45:11 *45:12 | *07.225 13.86 35.02.01.01_35.02.07 35.02.09 |
| 13 | 300 pp | 900 BD | 40.12 | *07:225, 13:86, 35:02:01:01-35:02:07, 35:02:08*, 35:02:09-35:02:16, 35:81, 35:83, 35:95, 35:129N, 35:146, 35:154, 35:157, 35:162, 35:172, 35:182- 35:184, 35:211, 35:220, 35:258, 35:270, 35:273, 35:285, 35:309, 35:311, 35:323, 35:335, 35:339, 35:357, 35:361, 35:366-35:367, 35:372, 35:374, 35:377-35:379, 35:387, 35:391, 35:394, 35:396, 35:403, 35:407, 35:410, 35:419, 35:443, 35:468, |

HLA-B*45

Product Insert

101.564-06 – including *Taq* polymerase, IFU-01 101.564-06u – without *Taq* polymerase, IFU-02 Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

| | Lot No.: 0M7 | b.: OM7 Lot-specific information | | | | | | | | | | | |
|------------------------|---------------------|---|------------------|--|--|--|--|--|--|--|--|--|--|
| | | | | 35:483, 35:487, 35:492, 50:36, 53:19, 53:36, 58:65 | | | | | | | | | |
| 14 | 200 bp | 1070 bp | *45:13 | | | | | | | | | | |
| 15 | 205 bp | 1070 bp | *45:09, 45:14 | *07:20, 07:140, 07:153, 15:14, 15:91, 15:131, 15:161, 15:518, 15:551, 18:56, 18:169, 35:45, 35:71, 35:169, 35:309, 35:452, 38:158, 40:132, 40:178, 44:10, 44:14, 44:17, 44:31, 44:43:01- 44:43:02, 44:45, 44:51, 44:62, 44:77, 44:82, 44:107, 44:123, 44:135, 44:184, 44:213, 44:230, 44:339, 46:17, 51:23, 51:108, 52:15, 53:22, 56:74, 57:07, 57:93, 57:119, 58:07, 82:01:01:01-82:03 | | | | | | | | | |
| 16 ⁵ | - | - | 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*45 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.

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.

³For several HLA Class I alleles 1st and/or 4th 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.

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

⁵Primer mix 16 contains a negative control, which will amplify the majority 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 200 base pairs.

'w', may be weakly amplified.

101.564-06 – including *Taq* polymerase, IFU-01 101.564-06u – without *Taq* polymerase, IFU-02 Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

Lot No.: **0M7**

Lot-specific information PRIMER SPECIFICATION

| Well No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|------------|----------------------------------|----------------------------------|
| Length of spec. | 255 | 150 | 160 | 160 | 195 | 255 | 230 | 105 | 245 | 160 | 245 | 205 |
| PCR product | | | | | | | | | | | | |
| Length of int. | 800 | 1070 | 800 | 800 | 800 | 1070 | 1070 | 1070 | 1070 | 1070 | 1070 | 1070 |
| pos. control ¹ | | | | | | | | | | | | |
| 5'-primer(s) ² | 357 | 206 | 420 | 416 | 420 | 357 | 357 | 206 | 357 | 419 | 357 | 357 |
| • • • • | ^{5'} -Tgg ^{3'} | ^{5'} -gAA ^{3'} | ^{5'} -TTA ^{3'} | 5' -CCg 3' | 5' -TTC 3' | ^{5'} -Tgg ^{3'} | ^{5'} -Tgg ^{3'} | ^{5'} -gAA ^{3'} | ^{5'} -Tgg ^{3'} | 5' -gTC 3' | ^{5'} -Tgg ^{3'} | ^{5'} -Tgg ^{3'} |
| | | | | 662 | | | | | | | | |
| | | | | ^{5'} -CCg ^{3'} | | | | | | | | |
| 3'-primer(s) ³ | 572 | 317 | 538 | 538 | 572 | 572 | 545 | 272 | 559 | 538 | 559 | 523 |
| (-/ | ^{5'} -gCg ^{3'} | 5' -CgC 3' | 5' -gTC 3' | 5' -gTC 3' | ^{5'} -gCg ^{3'} | 5' -gCC 3' | ^{5'} -AgA ^{3'} | ^{5'} -TgT ^{3'} | 5' -CgT ^{3'} | 5' -gTC 3' | 5' -CTC 3' | 5' -ACA 3 |
| | | | | 774 | | | | | | | | |
| | | | | ^{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. | 300 | 200 | 205 |
| PCR product | | | |
| | | | |
| Length of int. | 800 | 1070 | 1070 |
| pos. control ¹ | | | |
| 5'-primer(s) ² | 397 | 850 | 409 |
| | 5' -gCT 3' | 5' -gCT 3' | 5' -ggC 3' |
| | | | |
| 3'-primer(s) ³ | 3 rd I | 916 | 572 |
| | ^{5'} -TAT ^{3'} | ^{5'} -gAT ^{3'} | ^{5'} -gCg ^{3'} |
| Well No. | 13 | 14 | 15 |

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 <u>www.ebi.ac.uk/imgt/hla</u> 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 <u>www.ebi.ac.uk/imgt/hla</u> web site. The sequence of the 3 terminal nucleotides of the primer is given.

Product Insert

Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

101.564-06 – including *Taq* polymerase, IFU-01 101.564-06u – without *Taq* polymerase, IFU-02

Lot No.: **0M7**

Lot-specific information

| | | CE | | NE VA | LI | DA | ١T | 10 | Ν | Sł | ΗE | E | Г | | | | | | |
|----------|------|---------------------------|--------|-----------|-----------|-----------|-----------|-----------|-----------|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|----------|
| | | | HLA | -B*45 S | SS | Ρp | orir | ne | r s | et ² | | | | | | | | | |
| | | | | | | | | | ١ | Ne | | | | | | | | | |
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| | | | | | ~ | N | e | 4 | 2 | G | ~ | ø | 0 | 0 | ~ | 2 | e | 4 | 5 |
| | | | | 0 | 201901501 | 202129802 | 201901503 | 201901504 | 201901505 | 201901506 | 201901507 | 201901508 | 201901509 | 201901510 | 201901511 | 201901512 | 201901513 | 201901514 | |
| | | | | 2 | 901 | 126 | 901 | 901 | 901 | 901 | 901 | 901 | 901 | 901 | 901 | 901 | 901 | 901 | 901 |
| | | | | Prod. No. | 5 | 02 | 5 | 01 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 20190151 |
| | IHW | /C cell line ¹ | | B* | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| 1 | 9001 | | *07:02 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | | LK707 | *52:01 | *73:01 | - | - | - | - | - | + | - | - | - | - | + | - | - | - | - |
| 3 | | E4181324 | *52:01 | | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| 4 | | GU373 | *15:10 | *53:01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | | KAS011 | *37:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | 9353 | SM | *39:01 | *51:01 | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| 7 | 9020 | | *18:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 8 | 9025 | DEU | *35:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 9 | 9026 | YAR | *38:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 10 | 9107 | | *54:01 | | - | - | - | - | - | + | - | - | + | - | - | - | - | - | - |
| 11 | 9051 | PITOUT | *44:03 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 12 | 9052 | | *57:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 13 | 9004 | JESTHOM | *27:05 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 14 | | OLGA | *15:01 | *15:20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 15 | 9075 | | *40:01 | | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 16 | | SWEIG007 | *40:02 | | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 17 | | CTM3953540 | *08:01 | *55:01 | - | - | - | - | • | + | - | - | + | - | - | • | - | - | - |
| 18 | | 32367 | *14:01 | *56:01 | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| 19 | | BM16 | *18:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 20 | _ | SLE005 | *40:01 | | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 21 | | AMALA | *15:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 22 | | KOSE | *35:03 | *50.00 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 23 24 | 9124 | JBUSH | *40:02 | *56:02 | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 24 | 9035 | | *14:02 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 26 | | WT49 | *58:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 20 | | CH1007 | *07:05 | *51:01 | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| 28 | | BEL5GB | *44:02 | *44:03 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 29 | 9050 | | *44:03 | 11.00 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 30 | 9021 | | *42:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 31 | | DUCAF | *18:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 32 | 9297 | | *41:02 | | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 33 | | 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 | | *15:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 39 | 9315 | | *08:01 | *27:05 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 40 | | WHONP199 | *13:02 | *46:01 | - | - | - | - | - | + | - | - | - | - | + | - | - | - | - |
| 41 | | H0301 | *14:02 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 42 | | TAB089 | *46:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 43 | | T7526 | *46:01 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 44 | 9057 | | *38:01 | *50.01 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 45 | | SHJO | *42:01 | *50:01 | - | + | - | - | - | + | - | - | - | - | - | - | - | - | - |
| 46 | | SCHU | *07:02 | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 47 | | | *51:01 | *44.00 | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| 48 | 9303 | TER-ND | *35:01 | *44:03 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |



Product Insert

101.564-06 – including *Taq* polymerase, IFU-01 101.564-06u – without *Taq* polymerase, IFU-02

Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

Lot No.: 0M7

Lot-specific information

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

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 solutions 4, 5, 7, 8, 10, 12 and 14 were available. The specificities of the primers in primer solutions 5, 8 and 10 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer mixes 4 and 14 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer mixes 7 and 12 it was only possible to test the 5'-primer, the 3'-primer was not possible to test.



Product Insert

101.564-06 – including *Taq* polymerase, IFU-01 101.564-06u – without *Taq* polymerase, IFU-02 Visit <u>https://labproducts.caredx.com</u> for "Instructions for Use" (IFU)

Lot No.: **0M7**

Lot-specific information

ADDRESSES:

Manufacturer: CareDx AB, Franzengatan 5, SE-112 51 Stockholm, Sweden. Tel: +46-8-508 939 00 Fax: +46-8-717 88 18 E-mail: orders-se@caredx.com Web page: https://labproducts.caredx.com/

CareDx Lab Solutions Inc., 901 S. Bolmar St., Suite R, West Chester, PA 19382 Tel: 1-877-653-78171 Fax: 610-344-7989 E-mail: orders-us@caredx.com Web page: https://labproducts.caredx.com/

For information on CareDx distributors worldwide, contact CareDx AB.