OLERUP SSP

HLA-A\*25 Product Insert Page 1 of 12
101.423-06 – including *Taq* polymerase, IFU-01 Visit <a href="https://labproducts.caredx.com">https://labproducts.caredx.com</a> for

**101.423-06u – without** *Taq* **polymerase**, IFU-02

"Instructions for Use" (IFU)

Lot No.: 3N0 Lot-specific information

Olerup SSP® HLA-A\*25

Product number: 101.423-06 – including *Tag* polymerase

101.423-06u - without *Taq* polymerase

Lot number: 3N0

Expiry date: 2025-09-01

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. 3NO.

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-A\*25 Lot (6K4)

- The product documentation has been updated for new alleles of IMGT 3.44.0.
- The kit resolution focuses on common and well documented (CWD) alleles<sup>1</sup>.

The HLA-A\*25 primer set, specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup* SSP® HLA-A\*25 lot was made (Lot No. 6K4).

The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot (Lot No. 6K4).

Well	5'-primer	3'-primer	rationale
1	Added	-	5'-primer added for the A*25:01:17 allele.



<sup>&</sup>lt;sup>1</sup>As described in section Uniquely Identified Alleles.

**HLA-A\*25** 

**Product Insert** 

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**101.423-06 – including** *Taq* **polymerase**, IFU-01 **101.423-06u – without** *Taq* **polymerase**, IFU-02

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Lot No.: 3N0 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 <sup>1</sup>	164	340	440	45	45	43	36
	5'-CAC3'	<sup>5'</sup> -Agg <sup>3'</sup>	<sup>5'</sup> -TTA3'	<sup>5</sup> '-Tgg <sup>3</sup> '	<sup>5</sup> '-Tgg <sup>3</sup> '	<sup>5</sup> '-Tgg <sup>3</sup> '	<sup>5'</sup> -TAC <sup>3'</sup>
							36
							<sup>5'</sup> -TAT <sup>3'</sup>
3'-primer <sup>2</sup>	231	2 <sup>nd</sup> I	507	59	58	57	47
•	<sup>5</sup> '-TgC <sup>3</sup> '	<sup>5'</sup> -AAA <sup>3'</sup>	<sup>5'</sup> -TTg <sup>3'</sup>	<sup>5'</sup> -CTC <sup>3'</sup>	<sup>5'</sup> -ggC <sup>3'</sup>	5'-CTC <sup>3'</sup>	5'-ACA3'
							48
							<sup>5'</sup> -gCA <sup>3'</sup>
							48
							<sup>5'</sup> -gCC <sup>3'</sup>
							52
							<sup>5'</sup> -TgT <sup>3'</sup>
<b>A</b> *	+	+	+				
B*	+	+	+				
C*	+	+	+				
DRB1				+	+		
DRB3				+	+		
DRB5				+			
DQB1					+		
DPB1						+	
DQA1							+

<sup>&</sup>lt;sup>1</sup>The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2<sup>nd</sup> or 3<sup>rd</sup> exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and codon numbering as on the <a href="https://www.ebi.ac.uk/imgt/hla">www.ebi.ac.uk/imgt/hla</a> web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>&</sup>lt;sup>2</sup>The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2<sup>nd</sup> or 3<sup>rd</sup> exon or the 2<sup>nd</sup> intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and codon numbering as on the <a href="https://www.ebi.ac.uk/imgt/hla">www.ebi.ac.uk/imgt/hla</a> web site. The sequence of the 3 terminal nucleotides of the primer is given.



•LERUP SSP°

Lot No.: 3NO

HLA-A\*25 Product Insert

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**101.423-06 – including** *Taq* **polymerase**, IFU-01 **101.423-06u – without** *Taq* **polymerase**, IFU-02

Lot-specific information

### PRODUCT DESCRIPTION

### **HLA-A\*25 SSP subtyping**

#### CONTENT

The primer set contains 5'- and 3'-primers for identifying the A\*25:01 to A\*25:73 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	NC

The 16 well PCR plate is marked with 'HLA-A\*25' in silver/gray ink.

Well No. 1 is marked with the Lot No. '3N0'.

Wells 1 to 15 – HLA-A\*25 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-A alleles non-HLA-A\*25 alleles will be amplified by some primer mixes. For further details see Specificity Table.

#### **UNIQUELY IDENTIFIED ALLELES**

All the HLA-A\*25 alleles, i.e. **A\*25:01 to A\*25:73**, recognized by the HLA Nomenclature Committee in April 2021<sup>1,2</sup> will be amplified by the primers in the HLA-A\*25 subtyping kit<sup>3</sup>.

The HLA-A\*25 kit enables separation of the confirmed HLA-A\*25 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-A\*25 alleles is listed below.

The HLA-A\*25 kit also enables identification of many null and alternatively expressed alleles.



HLA-A\*25 Product Insert

**101.423-06 – including** *Taq* **polymerase**, IFU-01 **101.423-06u – without** *Taq* **polymerase**, IFU-02

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Lot No.: 3N0 Lot-specific information

<sup>1</sup>HLA-A alleles listed on the IMGT/HLA web page 2021-April-20, release 3.44.0, www.ebi.ac.uk/imgt/hla.

<sup>2</sup>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 <a href="http://hla.alleles.org/alleles/deleted.html">http://hla.alleles.org/alleles/deleted.html</a>.

<sup>3</sup>The HLA-A\*25 primer set cannot separate the following alleles. These alleles can be distinguished by the HLA-A low resolution kit and/or the respective high-resolution kits:

#### Alleles

A\*25:36, 25:43, 25:50, 25:52, 25:65, A\*32:62

A\*25:59, A\*01:315

A\*25:64, A\*32:26:01-32:26:02

#### **ALLELE CONFIRMATION STATUS**

Allele	Status <sup>1</sup>	Allele	Status <sup>1</sup>	Allele	Status <sup>1</sup>
A*25:01:01	Confirmed	A*25:13	Unconfirmed	A*25:31	Unconfirmed
A*25:01:02	Unconfirmed	A*25:14	Unconfirmed	A*25:32	Unconfirmed
A*25:01:03	Confirmed	A*25:15	Unconfirmed	A*25:33	Unconfirmed
A*25:01:04	Unconfirmed	A*25:16	Confirmed		
A*25:01:05	Unconfirmed	A*25:17	Unconfirmed		
A*25:01:06	Confirmed	A*25:18	Unconfirmed		
A*25:01:07	Unconfirmed	A*25:19:01	Confirmed		
A*25:01:08	Unconfirmed	A*25:19:02	Unconfirmed		
A*25:01:09	Unconfirmed	A*25:20	Unconfirmed		
A*25:02	Confirmed	A*25:21	Unconfirmed		
A*25:03	Confirmed	A*25:22	Unconfirmed		
A*25:04	Unconfirmed	A*25:23	Unconfirmed		
A*25:05	Unconfirmed	A*25:24	Confirmed		
A*25:06	Unconfirmed	A*25:25	Unconfirmed		
A*25:07	Unconfirmed	A*25:26	Unconfirmed		
A*25:08	Confirmed	A*25:27:01	Unconfirmed		
A*25:09	Confirmed	A*25:27:02	Confirmed		
A*25:10	Unconfirmed	A*25:28	Unconfirmed		
A*25:11	Confirmed	A*25:29	Unconfirmed		
A*25:12N	Confirmed	A*25:30	Unconfirmed		

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

#### **RESOLUTION IN HOMO- AND HETEROZYGOTES**

Results file with resolution in HLA-A\*25 homo- and heterozygotes is available upon request.





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101.423-06 – including *Taq* polymerase, IFU-01 Visit <a href="https://labproducts.caredx.com">https://labproducts.caredx.com</a> for 101.423-06u – without *Taq* polymerase, IFU-02 "Instructions for Use" (IFU)

Lot No.: 3N0 Lot-specific information

## **SPECIFICITY TABLE**

## **HLA-A\*25 SSP subtyping**

## Specificities and sizes of the PCR products of the 15+1 primer mixes used for HLA-A\*25 SSP subtyping

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA- A*25 alleles <sup>3</sup>	Other amplified HLA-A alleles
1 <sup>5</sup>	185 bp	800 bp	*25:01:01:01- 25:05, 25:07- 25:35, 25:37- 25:42N, 25:44- 25:49N, 25:51, 25:53-25:58, 25:60-25:64, 25:66-25:73	*02:135, 11:116, 11:140, 26:01:01:01-26:03:01:02, 26:05-26:08:03, 26:10-26:28, 26:30-26:43:02, 26:45-26:48, 26:50-26:74, 26:76-26:77, 26:79-26:90, 26:92-26:203, 26:206N-26:214, 31:24-31:25, 32:26:01-32:26:02, 33:61, 33:190, 43:01-43:02N, 66:01:01:01-66:01:05, 66:04-66:09, 66:11-66:15, 66:17-66:20, 66:22-66:24, 66:27N, 66:29-66:33, 66:35-66:38, 66:41-66:42, 74:37
2 <sup>4,6,8</sup>	75 bp	800 bp	*25:01:01:01- 25:14, 25:16, 25:18-25:19:01, 25:20-25:45, 25:47-25:58, 25:60-25:70, 25:72-25:73	*01:298, 02:829, 32:01:01:01- 32:01:13, 32:01:15-32:02, 32:04, 32:06-32:37, 32:39-32:59, 32:61- 32:70, 32:72-32:77, 32:81-32:92N, 32:94-32:106:01:02, 32:108-32:151
34	100 bp	800 bp	*25:01:01- 25:01:20, 25:03- 25:12N, 25:14, 25:16, 25:18- 25:63, 25:65- 25:70, 25:72- 25:73	*01:01:01:01-01:01:27, 01:01:29-01:01:56, 01:01:58-01:01:61, 01:01:63-01:01:106, 01:01:108-01:01:123, 01:01:125-01:04:01:02N, 01:06, 01:08-01:12, 01:14-01:16N, 01:18N-01:33, 01:35-01:70, 01:72-01:99, 01:101-01:104, 01:106-01:143, 01:145-01:175, 01:177-01:189, 01:191-01:193, 01:195-01:203, 01:205-01:249, 01:251-01:288, 01:290N-01:297, 01:299-01:301Q, 01:303:01-01:325, 01:327-01:333, 01:335-01:377, 02:346, 02:427, 02:644, 02:749, 02:952, 03:41, 11:17, 11:19, 11:40, 11:98, 11:121, 11:308, 11:312, 24:44*, 24:109*, 24:260*, 26:01:01:01-26:01:21, 26:01:23-26:01:27, 26:01:29-26:12, 26:14-26:18, 26:20:01-26:29, 26:31-26:32, 26:34-26:43:02, 26:45-26:63, 26:66-26:82, 26:84-26:131, 26:133-26:147, 26:149-26:150, 26:152-26:193, 26:196-26:214, 31:03, 31:164, 32:62, 33:13, 33:184, 36:01:01:01-36:12,



HLA-A\*25 Product Insert 101.423-06 – including *Taq* polymerase, IFU-01 101.423-06u – without *Taq* polymerase, IFU-02

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Lot No.: 3	110		ot-specific informat	
				43:01-43:02N, 66:05, 66:15, 74:10, 74:30, 80:01:01:01-80:09N
44	90 bp	800 bp	*25:02, 25:24	*01:13, 01:17, 01:176, 01:302, 02:741, 03:63, 03:88, 03:382, 11:01:01:01-11:01:67, 11:01:69-11:01:72, 11:01:74-11:01:75, 11:01:77-11:11, 11:13-11:16, 11:20-11:27, 11:29-11:39, 11:41-11:52Q, 11:54:01-11:95, 11:97, 11:99N-11:105, 11:107-11:120, 11:122-11:158, 11:160-11:177, 11:179-11:249, 11:251N-11:290, 11:292-11:300, 11:302:01-11:307, 11:309-11:311, 11:313Q-11:324, 11:326-11:398, 26:13, 26:19, 26:33, 29:66, 30:125, 30:140, 34:01:01:01-34:06, 34:08, 34:10N-34:26N, 66:01:01:01-66:01:05, 66:04, 66:06-66:11, 66:13-66:14, 66:17-66:20, 66:22-66:24, 66:27N, 66:29-66:33, 66:35-66:38, 66:40-66:42, 68:227, 69:02, <b>C*07:335</b>
5 <sup>6</sup>	425 bp	1070 bp	*25:02, 25:59	*01:315, 02:34-02:35:03, 02:56:01-02:56:02, 02:62, 02:78, 02:103, 02:580, 02:609, 02:741, 02:907, 03:242, 03:312, 23:09, 23:13, 24:07:01:01-24:07:04, 24:19, 24:24, 24:112, 24:129, 24:131, 24:288, 24:290, 24:294Q, 24:339, 24:347:01-24:347:02, 24:387, 24:406, 24:453, 24:457, 24:477, 24:489, 24:510, 24:528, 26:13, 26:19, 26:62, 26:72, 29:155, 34:01:01:01-34:13, 34:15-34:26N, 66:01:01:01-66:02, 66:04, 66:06-66:14, 66:16-66:27N, 66:29-66:38, 66:40-66:43, 68:01:01:01-68:02:19, 68:06-68:14, 68:16-68:19, 68:21:01-68:30, 68:32-68:56, 68:58:01-68:89, 68:91-68:108, 68:110-68:179, 68:181N-68:205N, 68:207-68:248, 68:250-68:254, 68:256-68:265, 69:01:01:01-69:08
6	155 bp	800 bp	*25:19:01- 25:19:02, 25:30	*02:309, 02:454, 03:01:19, 03:103:02, 26:43:01-26:43:02, 26:112, 31:03-31:04:02, 31:123, 34:02:01:01-34:04, 34:06-34:09, 34:13, 34:15, 34:20-34:22, 34:24-34:26N, 66:06, 66:33, 66:43, 74:01:03
	200 bp		*25:03, 25:30	*01:51, 02:55, 02:644, 02:741, 02:815, 03:24, 26:20:01-26:20:02, 32:15, 34:08, 68:71, 69:07
7	165 bp	800 bp	*25:04, 25:06, 25:12N, 25:19:01- 25:19:02, 25:30, 25:42N, 25:44	*02:309, 02:454, 03:01:19, 03:103:02, 26:08:01:01-26:09, 26:43:01-26:43:02, 26:48, 26:67, 26:73, 26:81, 26:86, 26:91, 26:112, 26:124, 26:146, 26:150, 26:189, 31:03-31:04:02, 31:123,



HLA-A\*25 Product Insert

**101.423-06 – including** *Taq* **polymerase**, IFU-01 **101.423-06u – without** *Taq* **polymerase**, IFU-02

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34:01:01:01-34:09, 34:12 66:06, 66:33, 66:43, 74:0	•
<b>8</b> <sup>4,6</sup> 100 bp 1070 bp *25:16 125 bp *25:05 *02:454, 26:54 165 bp *25:12N 235 bp *25:27:01- *26:130, 66:14 25:27:02	
<b>9</b> <sup>4</sup> 100 bp 1070 bp *25:16 135 bp *25:08 *26:47, 66:18 170 bp *25:42N 230 bp *25:49N	
<b>10</b> <sup>5,7</sup> 170 bp 1070 bp *25:07 230 bp *25:27:01- *26:130, 66:14 25:27:02, 25:49N	
11 <sup>6</sup> 135 bp 1070 bp *25:09 *03:01:19, 03:103:02, 26 26:28, 26:73, 26:112, 26: 31:04:02, 31:123, 34:03, 66:22, 66:43, 74:01:03	146, 31:03-
12 <sup>4</sup> 75 bp 1070 bp *25:10 *02:309, 02:454, 03:01:1 26:176, 31:03-31:04:02, 34:04, 34:07-34:10N, 34: 34:20-34:22, 34:24-34:26	34:02:01:01- 13, 34:15,
<b>13</b> <sup>4</sup> 65 bp 1070 bp *25:11 *23:12, 24:30, 24:42, 24: 32:08	309, 24:379,
14 <sup>4</sup> 80 bp 1070 bp *25:01:01:01- *32:62 25:01:03-25:12N, 25:14-25:32, 25:34-25:54, 25:60-25:63, 25:65-25:73	
15 360 bp 1070 bp *25:13 *02:35, 02:309, 02:454, 03:103:02, 26:30, 26:65, 31:04:02, 31:123, 34:09, 66:03:01:03, 66:12, 66:10, 66:25-66:26Q, 66:28N, 66:43, 74:01:03	31:04:01:01- 66:02- 6, 66:21,
16 <sup>9</sup> - Negative Control	

<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-A\*25 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.



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**101.423-06 – including** *Taq* **polymerase**, IFU-01 **101.423-06u – without** *Taq* **polymerase**, IFU-02

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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.

<sup>2</sup>The 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.

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

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

<sup>5</sup>Primer mixes 1 and 10 may give rise to a PCR fragment approx. 500 bp in size. This band should be disregarded in the interpretation of HLA-A\*25 subtypings.

<sup>6</sup>Primer mixes 2, 5, 8 and 11 may have tendencies of unspecific amplifications.

<sup>7</sup>Primer mix 10 might have a tendency for primer oligomer formation.

<sup>8</sup>Primer mix 8 may weakly amplify A\*34 alleles.

<sup>9</sup>Primer 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.

Abbreviations

w: may be weakly amplified

**HLA-A\*25** 

**Product Insert** 

**101.423-06 – including** *Taq* **polymerase**, IFU-01 **101.423-06u – without** *Taq* **polymerase**, IFU-02

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Lot No.: 3NO Lot-specific information

## PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec.	185	75	100	90	425	155	165	100	100	170	135	75
PCR product						200		125	135	230		
								165	170			
								235	230			
Length of int.	800	800	800	800	1070	800	800	1070	1070	1070	1070	1070
pos. control <sup>1</sup>												
5'-primer(s) <sup>2</sup>	414	266	282	282	28	102	423	423	423	423	423	385
	<sup>5'</sup> -CAg <sup>3'</sup>	5' -ACg 3'	5' -CAC 3'	5' -CAg 3'	<sup>5'</sup> -TCg <sup>3'</sup>	5' -ACA 3'	<sup>5'</sup> -gCT <sup>3'</sup>	<sup>5'</sup> -gCT <sup>3'</sup>	<sup>5'</sup> -gCT <sup>3'</sup>	<sup>5'</sup> -gCT <sup>3'</sup>	5' -gCT 3'	<sup>5'</sup> -ggT <sup>3'</sup>
	418	266		296		423				650		
	<sup>5'</sup> -AgA <sup>3'</sup>	5' -ACg 3'		5' -CCC 3'		5' -gCT 3'				5' -Cgg 3'		
	418											
	5' -Agg 3'											
	423											
	<sup>5'</sup> -gCT <sup>3'</sup>											
3'-primer(s) <sup>3</sup>	560	302	341	341	271	259	538	484	484	610	517	418
	5' -ACC 3'	<sup>5'</sup> -ggC <sup>3'</sup>	5' -CgT 3'	5' -CgT 3'	5' -CAT 3'	<sup>5'</sup> -gTT <sup>3'</sup>	<sup>5'</sup> -CTg <sup>3'</sup>	5' -CAg 3'	5' -CAg 3'	5' -CTA 3'	<sup>5'</sup> -CgC <sup>3'</sup>	<sup>5'</sup> -gTC <sup>3'</sup>
					282	539	539	506	518	616		
					5' -gAC 3'	5' -TCA 3'	5' -TCA 3'	<sup>5'</sup> -TgT <sup>3'</sup>	5' -CCA 3'	5' -CgC 3'		
					282		549	549	553	616		
					5' -gAC 3'		<sup>5'</sup> -AgT <sup>3'</sup>	<sup>5'</sup> -AgT <sup>3'</sup>	5' -CTA 3'	<sup>5'</sup> -TgC <sup>3'</sup>		
							553	616	610	777		
							5' -CTA 3'	<sup>5'</sup> -CgC <sup>3'</sup>	5' -CTA 3'	<sup>5'</sup> -gCA <sup>3'</sup>		
							559	616				
							<sup>5'</sup> -CgT <sup>3'</sup>	<sup>5'</sup> -TgC <sup>3'</sup>				
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15
Length of spec.	65	80	360
PCR product			
Length of int.	1070	1070	1070
pos. control <sup>1</sup>			
5'-primer(s) <sup>2</sup>	292	302	341
	5' -CTC 3'	<sup>5'</sup> -gAg <sup>3'</sup>	<sup>5'</sup> -ggC <sup>3'</sup>
3'-primer(s) <sup>3</sup>	317	341	418
	<sup>5'</sup> -ggA <sup>3'</sup>	<sup>5'</sup> -CgT <sup>3'</sup>	<sup>5'</sup> -gTC <sup>3'</sup>
Well No.	13	14	15

<sup>1</sup>The 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

<sup>2</sup>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.

<sup>3</sup>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.





HLA-A\*25

**Product Insert** 

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**101.423-06 – including** *Taq* **polymerase**, IFU-01 **101.423-06u – without** *Taq* **polymerase**, IFU-02

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Lot No.: 3N0 Lot-specific information

LOT IN	CELL LINE VALIDATION SHEET																		
			HLA-	A*25 S	SP	, Sι	ıbt	ур	inç	j k	it <sup>2</sup>								
												Vе	II						
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
					_	2	3	4	5	9	7	8	6	0	_	2	3	4	5
					20213240	201667102	201667103	201667104	201913705	201890306	201890307	201667108	201903609	201903610	201667111	201667112	201667113	201667114	201903615
				Prod No.	13	.99	.99	.99	91;	89	89	.99	90	90	.99	.99	.99	.99	903
				l or	182	201	201	201	201	201	201	201	201	201	201	20	20	201	201
	IHV	/C cell line <sup>1</sup>	A*	<b>A</b> *					.,		.,								.,
1	9001		*24:02		+-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2		LK707	*02:01		T -	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3		E4181324	*01:01		1 -	-	+	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*30:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*01:01		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*02:01	*26:03	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*26:01		+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
8	9025	DEU	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*26:01		+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
10	9107		*24:02		<u> </u>	<u> </u>	-	-		<u> </u>	<u> </u>	-	-	-	-	<u> </u>	<u> </u>	-	-
11		PITOUT	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052		*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13		JESTHOM	*02:01		ļ-	-	-	-	-	-	-	-	-	-	-		-	-	-
14		OLGA	*31:01		ļ-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075		*24:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16		SWEIG007	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17		CTM3953540	*03:01	*80:01	-	-	+	-	-	-	-	-	-	-	-		-	-	-
18		32367	*33:03	*74:01	+-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19		BM16	*02:01		+-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20		SLE005	*02:01		+-	<u> </u>	·	-	-	-	<u> </u>	Ŀ	-	-	Ŀ	-	-	·	-
21		AMALA KOSE	*02:17 *02:01		+-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124		*02:01	*34:01	╀	H	H	-		H		÷	Ë	-	÷	-	H	-	-
24		JBUSH	*32:01	34.01	+:	+	H	+	+	H	+	-	H	-	÷	-	H	E	-
25	9049		*33:01		+-	T	-	-	-	-	-	-	-	-	-	-	-	-	-
26		WT49	*02:05		١.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27		CH1007	*24:10	*29:01	1-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28		BEL5GB	*02:01	*29:02	1-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050		*29:02	20.02	1-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021		*30:01	*68:02	1 -	-	-	-	+	-	-	-	-	-	-	-	-	-	-
31		DUCAF	*30:02		1-	-	-	-	Ė	-	-	-	-	-	-	-	-	-	-
32	9297		*02:01		1 -	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33		MT14B	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104		*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*32:01		-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*02:06	*11:01	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099		*02:17		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315		*01:01	*03:01	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
40		WHONP199	*02:07	*30:01	<u> </u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41		H0301	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42		TAB089	*02:07		ļ-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43		T7526	*02:06	*02:07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057		*66:01		+	-	-	+	+	-	-	-	-	-	-	-	-	-	-
45		SHJO	*23:01	*24:02	<b>!</b> -	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46		SCHU	*03:01	*00.01	<b>!</b> -	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47		TUBO	*02:16	*03:01	<b>!</b> -	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*02:01	*11:01		-	-	+	-	-	-	-	-	-	-	-	-	•	-

**●LERUP SSP** 

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101.423-06 – including *Taq* polymerase, IFU-01 Visit <a href="https://labproducts.caredx.com">https://labproducts.caredx.com</a> for 101.423-06u – without *Taq* polymerase, IFU-02 "Instructions for Use" (IFU)

Lot No.: **3N0** Lot-specific information

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

<sup>2</sup>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 alleles to be amplified by primer solutions 8 to 11 and 13 were available. The specificities of the primers in primer solutions 8, 10, 11 and 13 were tested by separately adding one 5'-primer, and one I 3'-primer accordingly. In primer solution 9 it was only possible to test the 5'-primer, the 3'-primers were not possible to be tested.

In primer solutions 4 and 10 one 5'-primer was not possible to be tested, and in primer solutions 7, 8 and 10 two to four 3'-primers were not possible to be tested.

In addition, one or more 5'- and/or 3'-primers in primer solutions 6 and 7 were tested by separately adding either one 3'-primer or/and one 5'-primer respectively.

•LERUP SSP°

HLA-A\*25 Product Insert Page 12 of 12
101.423-06 – including *Taq* polymerase, IFU-01 Visit <a href="https://labproducts.caredx.com">https://labproducts.caredx.com</a> for 101.423-06u – without *Taq* polymerase, IFU-02 "Instructions for Use" (IFU)

Lot No.: 3N0 Lot-specific information

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