

101.430-12 – including *Taq* polymerase, IFU-01  
101.430-12u – without *Taq* polymerase, IFU-02

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“Instructions for Use” (IFU)

Lot No.: **1D7**

Lot-specific Information  
**Olerup SSP® HLA-A\*31**

[www.olerup-ssp.com](http://www.olerup-ssp.com)

<b>Product number:</b>	<b>101.430-12 – including <i>Taq</i> polymerase</b> <b>101.430-12u – without <i>Taq</i> polymerase</b>
<b>Lot number:</b>	<b>1D7</b>
<b>Expiry date:</b>	<b>2018-May-01</b>
<b>Number of tests:</b>	<b>12</b>
<b>Number of wells per test:</b>	<b>31+1</b>
<b>Storage - pre-aliquoted primers:</b>	<b>dark at -20°C</b>
- PCR Master Mix:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

**This Product Description is only valid for Lot No. 1D7.**

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\*31 LOT (14X)**

The HLA-A\*31 kit is updated for new alleles to enable separation of:

- Confirmed<sup>1</sup> alleles as listed in the IMGT/HLA database
- Polymorphisms in exons outside of the region encoding the peptide binding domain
- Null and Alternatively expressed alleles

A well containing Negative Control primer pairs has been added.

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

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

The HLA-A\*31 primer set, specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP®* HLA-A\*31 lot was made (**Lot No. 14X**). The kit design is based on IMGT/HLA database 3.21.1.

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

Well	5'-primer	3'-primer	rationale
21	Added	-	5'-primer added for the A*31:01:02:03N allele.
24	-	Added	3'-primer added for the A*31:89 allele.
32	-	-	Updated negative control.

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Well **32** 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
<b>5'-primer<sup>1</sup></b>	<b>164</b>	<b>340</b>	<b>440</b>	<b>45</b>	<b>45</b>	<b>43</b>	<b>36</b>
	5'-CAC <sup>3'</sup>	5'-Agg <sup>3'</sup>	5'-TTA <sup>3'</sup>	5'-Tgg <sup>3'</sup>	5'-Tgg <sup>3'</sup>	5'-Tgg <sup>3'</sup>	5'-TAC <sup>3'</sup>
							<b>36</b>
							5'-TAT <sup>3'</sup>
<b>3'-primer<sup>2</sup></b>	<b>231</b>	<b>2<sup>nd</sup> I</b>	<b>507</b>	<b>59</b>	<b>58</b>	<b>57</b>	<b>47</b>
	5'-TgC <sup>3'</sup>	5'-AAA <sup>3'</sup>	5'-TTg <sup>3'</sup>	5'-CTC <sup>3'</sup>	5'-ggC <sup>3'</sup>	5'-CTC <sup>3'</sup>	5'-ACA <sup>3'</sup>
							<b>48</b>
							5'-gCA <sup>3'</sup>
							<b>48</b>
							5'-gCC <sup>3'</sup>
							<b>52</b>
							5'-TgT <sup>3'</sup>
<b>A*</b>	<b>+</b>	<b>+</b>	<b>+</b>				
<b>B*</b>	<b>+</b>	<b>+</b>	<b>+</b>				
<b>C*</b>	<b>+</b>	<b>+</b>	<b>+</b>				
<b>DRB1</b>				<b>+</b>	<b>+</b>		
<b>DRB3</b>				<b>+</b>	<b>+</b>		
<b>DRB5</b>				<b>+</b>			
<b>DQB1</b>					<b>+</b>		
<b>DPB1</b>						<b>+</b>	
<b>DQA1</b>							<b>+</b>

<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 [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<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 [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

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## PRODUCT DESCRIPTION

### HLA-A\*31 SSP subtyping

#### CONTENT

The primer set contains 5'- and 3'-primers for identifying the A\*31:01 to A\*31:98 alleles.

#### PLATE LAYOUT

Each test consists of 32 PCR reactions in a 32 well cut PCR plate.

<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>
<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>
<b>17</b>	<b>18</b>	<b>19</b>	<b>20</b>	<b>21</b>	<b>22</b>	<b>23</b>	<b>24</b>
<b>25</b>	<b>26</b>	<b>27</b>	<b>28</b>	<b>29</b>	<b>30</b>	<b>31</b>	<b>NC</b>

The 32 well cut PCR plate is marked with 'HLA-A\*31' in silver/gray ink.

Well No. 1 is marked with the Lot Number '1D7'.

Wells 1 to 31 – HLA-A\*31 high resolution primers.

Well 32 – 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 covered with a PCR-compatible foil.

**Please note:** When removing each 32 well PCR plate, make sure that the remaining plates stay covered. 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\*31 alleles will be amplified by primer mixes 1 to 19, 21 to 29 and 31.

In addition, a few HLA-B and HLA-C alleles will be amplified by primer mixes 1, 9, 25 and 26.

For further details see Specificity Table.

#### UNIQUELY IDENTIFIED ALLELES

All the HLA-A\*31 alleles, i.e. **A\*31:01 to A\*31:98 alleles**, recognized by the HLA Nomenclature Committee in August 2015<sup>1,2</sup> will be amplified by the primers in the HLA-A\*31 subtyping kit<sup>3</sup>.

The HLA-A\*31 kit enables separation of the confirmed HLA- A\*31 alleles as listed in the IMGT/HLA database. An HLA allele is listed as confirmed by IMGT/HLA if it has been sequenced by more than a single laboratory or from multiple sources. Current allele confirmation status for HLA- A\*31 alleles is listed below.

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The HLA-A\*31 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.

The following HLA-A\*31 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
A*31:01:02:03N, 31:35	21	A*31:29, 31:59	25
A*31:16, 31:46	16	A*31:36, 31:48	29

The HLA-A\*31 subtyping kit cannot distinguish the silent mutations in the A\*31:01:02:01-31:01:02:02 and 31:01:03-31:01:24 alleles.

<sup>1</sup>HLA-A alleles listed on the IMGT/HLA web page 2015-August-11, release 3.21.0, [www.ebi.ac.uk/imgt/hla](http://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 <http://hla.alleles.org/alleles/deleted.html>.

<sup>3</sup>The A\*31:44 and the A\*33:15 alleles can be distinguished by the different sizes of the specific PCR products generated by primer mix 29.

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## ALLELE CONFIRMATION STATUS

Allele	Status <sup>1</sup>	Allele	Status <sup>1</sup>	Allele	Status <sup>1</sup>	Allele	Status <sup>1</sup>
<b>A*31:01:02:01</b>	<b>Confirmed</b>	<b>A*31:17</b>	<b>Confirmed</b>	A*31:57	Unconfirmed	A*31:98	Unconfirmed
A*31:01:02:02	Unconfirmed	<b>A*31:18</b>	<b>Confirmed</b>	A*31:58	Unconfirmed		
A*31:01:02:03N	Unconfirmed	<b>A*31:19</b>	<b>Confirmed</b>	A*31:59	Unconfirmed		
A*31:01:03	Unconfirmed	<b>A*31:20</b>	<b>Confirmed</b>	A*31:60N	Unconfirmed		
<b>A*31:01:04</b>	<b>Confirmed</b>	A*31:21	Unconfirmed	A*31:61	Unconfirmed		
A*31:01:05	Unconfirmed	<b>A*31:22</b>	<b>Confirmed</b>	A*31:62	Unconfirmed		
A*31:01:06	Unconfirmed	A*31:23	Unconfirmed	A*31:63	Unconfirmed		
A*31:01:07	Unconfirmed	<b>A*31:24</b>	<b>Confirmed</b>	A*31:64	Unconfirmed		
A*31:01:08	Unconfirmed	A*31:25	Unconfirmed	A*31:65	Unconfirmed		
A*31:01:09	Unconfirmed	<b>A*31:26</b>	<b>Confirmed</b>	A*31:66	Unconfirmed		
A*31:01:10	Unconfirmed	<b>A*31:27</b>	<b>Confirmed</b>	A*31:67	Unconfirmed		
A*31:01:11	Unconfirmed	<b>A*31:28</b>	<b>Confirmed</b>	<b>A*31:68</b>	<b>Confirmed</b>		
A*31:01:12	Unconfirmed	A*31:29	Unconfirmed	A*31:69	Unconfirmed		
A*31:01:13	Unconfirmed	A*31:30	Unconfirmed	A*31:70	Unconfirmed		
A*31:01:14	Unconfirmed	<b>A*31:31</b>	<b>Confirmed</b>	A*31:71	Unconfirmed		
A*31:01:15	Unconfirmed	A*31:32	Unconfirmed	A*31:72	Unconfirmed		
A*31:01:16	Unconfirmed	A*31:33	Unconfirmed	A*31:73	Unconfirmed		
A*31:01:17	Unconfirmed	A*31:34	Unconfirmed	A*31:74	Unconfirmed		
A*31:01:18	Unconfirmed	A*31:35	Unconfirmed	A*31:75	Unconfirmed		
A*31:01:19	Unconfirmed	<b>A*31:36</b>	<b>Confirmed</b>	A*31:76	Unconfirmed		
A*31:01:20	Unconfirmed	A*31:37	Unconfirmed	A*31:77	Unconfirmed		
A*31:01:21	Unconfirmed	<b>A*31:38</b>	<b>Confirmed</b>	A*31:78	Unconfirmed		
A*31:01:22	Unconfirmed	<b>A*31:39</b>	<b>Confirmed</b>	A*31:79	Unconfirmed		
A*31:01:23	Unconfirmed	<b>A*31:40</b>	<b>Confirmed</b>	A*31:80	Unconfirmed		
A*31:01:24	Unconfirmed	<b>A*31:41</b>	<b>Confirmed</b>	A*31:81	Unconfirmed		
<b>A*31:02</b>	<b>Confirmed</b>	A*31:42	Unconfirmed	A*31:82	Unconfirmed		
A*31:03	Unconfirmed	<b>A*31:43</b>	<b>Confirmed</b>	A*31:83	Unconfirmed		
<b>A*31:04</b>	<b>Confirmed</b>	<b>A*31:44</b>	<b>Confirmed</b>	A*31:84	Unconfirmed		
<b>A*31:05</b>	<b>Confirmed</b>	A*31:45	Unconfirmed	A*31:85	Unconfirmed		
<b>A*31:06</b>	<b>Confirmed</b>	A*31:46	Unconfirmed	A*31:86	Unconfirmed		
A*31:07	Unconfirmed	A*31:47	Unconfirmed	A*31:87	Unconfirmed		
<b>A*31:08</b>	<b>Confirmed</b>	<b>A*31:48</b>	<b>Confirmed</b>	A*31:88	Unconfirmed		
<b>A*31:09</b>	<b>Confirmed</b>	A*31:49	Unconfirmed	A*31:89	Unconfirmed		
A*31:10	Unconfirmed	A*31:50	Unconfirmed	A*31:90	Unconfirmed		
<b>A*31:11</b>	<b>Confirmed</b>	A*31:51	Unconfirmed	A*31:91	Unconfirmed		
<b>A*31:12</b>	<b>Confirmed</b>	A*31:52	Unconfirmed	A*31:92	Unconfirmed		
A*31:13	Unconfirmed	A*31:53	Unconfirmed	A*31:93	Unconfirmed		
A*31:14N	Unconfirmed	<b>A*31:54</b>	<b>Confirmed</b>	A*31:94	Unconfirmed		
<b>A*31:15</b>	<b>Confirmed</b>	A*31:55	Unconfirmed	A*31:96	Unconfirmed		
<b>A*31:16</b>	<b>Confirmed</b>	<b>A*31:56</b>	<b>Confirmed</b>	A*31:97	Unconfirmed		

<sup>1</sup>Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2015-August-11, release 3.21.1, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

## RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in HLA-A\*31 homo- and heterozygotes is available upon request.

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Lot-specific Information  
**SPECIFICITY TABLE**

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**HLA-A\*31 SSP subtyping**

**Specificities and sizes of the PCR products of the 31+1 primer mixes used for HLA-A\*31 SSP subtyping**

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-A*31 alleles <sup>3</sup>	Other amplified HLA-A alleles <sup>4</sup>
<b>1</b>	155 bp	<b>800 bp</b>	*31:01:02:01-31:07, 31:09-31:43, 31:45-31:86, 31:88, 31:90-31:94, 31:96-31:98	*01:07, 02:185, 23:21, 24:124, 26:19, 29:14, 30:12, 30:18, 30:55, 34:04, <b>B*15:82</b> , <b>B*15:260</b> , <b>B*40:186</b> , <b>C*03:186:01</b>
<b>2<sup>5</sup></b>	80 bp  215 bp	<b>800 bp</b>	*31:67-31:68  *31:02, 31:07-31:08, 31:91	*02:41, 02:80, 02:117, 02:289:01, 02:304, 02:454, 23:45, 24:62, 26:10, 32:28, 32:66, 33:32:01 *02:243:01-02:243:02, 24:82, 29:48, 33:08, 33:53
<b>3</b>	155 bp	<b>800 bp</b>	*31:03-31:04	*02:309, 02:454, 03:01:19, 25:19:01-25:19:02, 25:30, 26:43:01, 34:02:01, 34:02:03-34:04, 34:06-34:09, 66:06
<b>4<sup>7</sup></b>	165 bp	1070 bp	*31:03-31:04, 31:06	*01:06, 02:114, 02:246, 02:279, 03:01:30, 03:05:01-03:05:02, 03:42, 03:98, 03:105, 03:122, 11:24:01-11:25:01, 11:31, 11:35, 11:158, 23:53, 29:01:01:01-29:01:02, 29:01:04-29:02:13, 29:02:15-29:04, 29:06-29:23, 29:25-29:27, 29:29-29:31, 29:34-29:76, 29:78N, 29:80, 30:26, 32:30, 32:32, 33:18:01-33:18:02, 34:02:01-34:04, 34:07-34:10N, 68:08:01-68:08:02, 68:63, 80:01:01:01-80:03
<b>5<sup>5</sup></b>	120 bp 285 bp	<b>800 bp</b>	*31:26, 31:38 *31:03	*03:184, 32:36 *01:143, 11:43, 29:66, 33:13
<b>6</b>	130 bp 165 bp	<b>800 bp</b>	*31:39, 31:54 *31:05	*33:07 *23:03:01, 24:21:03, 24:208, 29:03, 29:33, 32:13, 33:10
<b>7<sup>7</sup></b>	505 bp	1070 bp	*31:07-31:08, 31:10	*02:81, 02:87, 02:112, 02:124, 02:129, 03:152, 03:219, 23:01:01-23:01:15, 23:01:17-23:01:18, 23:03:01-23:13, 23:14:02-23:26, 23:28-23:33, 23:35-23:37:02, 23:39-23:65, 23:67-23:69, 24:13:01, 24:18, 24:24, 24:94, 24:188, 24:207, 24:228, 25:19:01-25:19:02, 25:30, 29:13, 32:01:01-32:01:05,

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				32:01:07-32:01:22, 32:03-32:09, 32:11Q-32:21, 32:23-32:70, 32:72-32:74
<b>8</b>	155 bp 220 bp	1070 bp	*31:24, 31:27 *31:09	*11:01:28, 29:67, 33:61
<b>9<sup>5</sup></b>	75 bp	1070 bp	*31:01:02:01-31:02, 31:05, 31:07-31:61, 31:63-31:66, 31:70- 31:94, 31:96-31:98	*02:24:02, 02:65, 02:152, 02:507, 23:03:01, 24:21:03, 29:32, 32:01:01-32:01:06, 32:01:08-32:01:11, 32:01:13- 32:03, 32:05-32:27N, 32:29, 32:31, 32:33:01, 32:34-32:47, 32:49-32:65, 32:67-32:74, 33:01:01-33:01:04, 33:01:06- 33:01:07, 33:03:01-33:03:18, 33:03:20-33:03:23, 33:03:25- 33:03:26, 33:03:28-33:17, 33:20-33:31, 33:33-33:37, 33:39-33:102, 74:01:01-74:24, <b>B*15:17:03</b>
<b>10<sup>5,6</sup></b>	115 bp	1070 bp	*31:41	*02:24:02, 02:507, 24:21:03, 24:208, 29:33, 29:51, 29:80, 32:02, 32:06, 33:94
	160 bp		*31:11, 31:56	
<b>11</b>	135 bp 210 bp	1070 bp	*31:26 *31:12, 31:60N	*03:184, 32:36 *02:490N, 02:516N, 02:526
<b>12</b>	245 bp	1070 bp	*31:01:02:01-31:06, 31:09, 31:11-31:20, 31:22-31:32, 31:34- 31:78, 31:80-31:94, 31:96-31:98	*02:243:01-02:243:02, 03:205, 11:43, 29:19, 29:39, 29:48, 33:01:01-33:01:07, 33:03:01- 33:12, 33:14-33:16, 33:18:01- 33:37, 33:39-33:47, 33:49- 33:50, 33:52-33:68, 33:70- 33:91, 33:93-33:102, 68:29
<b>13<sup>5</sup></b>	85 bp	1070 bp	*31:13	*02:251
<b>14<sup>8</sup></b>	150 bp 220 bp	<b>800 bp</b>	*31:24 *31:14N	*11:01:28, 29:67, 33:61
<b>15</b>	150 bp 225 bp	1070 bp	*31:25 *31:15	*32:26
<b>16<sup>5</sup></b>	80 bp 165 bp	1070 bp	*31:46 *31:16	*29:12, 33:58
<b>17</b>	160 bp 235 bp	1070 bp	*31:25, 31:40 *31:17	*32:26
<b>18</b>	155 bp 200 bp	1070 bp	*31:43, 31:62 *31:18	*02:408 *23:43
<b>19<sup>5</sup></b>	110 bp 185 bp	1070 bp	*31:19 *31:72	*02:380, 03:52
<b>20</b>	325 bp	1070 bp	*31:20	
<b>21<sup>5</sup></b>	75 bp 155 bp 180 bp 215 bp	1070 bp	*31:35 *31:43, 31:62 *31:21 *31:01:02:03N	*01:07 *02:408 *01:07, 02:185, 30:55



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<b>22<sup>5</sup></b>	80 bp	1070 bp	*31:71	*02:327
	155 bp		*31:27, 31:55	
	190 bp		*31:22	
<b>23<sup>5</sup></b>	80 bp	1070 bp	*31:71	*02:327
	165 bp		*31:40	
	200 bp		*31:23	
<b>24</b>	150 bp	1070 bp	*31:55	*02:104 *03:205, 11:43, 68:29
	180 bp		*31:81	
	220 bp		*31:28, 31:89	
<b>25<sup>6</sup></b>	135 bp	<b>800 bp</b>	*31:29	*02:507, 23:03:01, 24:21:03, 24:208, 29:07, <b>B*07:02:40,</b> <b>B*15:17:03</b>
	165 bp		*31:56	
	245 bp		*31:59	
<b>26</b>	130 bp	1070 bp	*31:30, 31:39, 31:97	*02:507, 29:28, 29:79, 32:10, 33:94, <b>B*07:02:40, C*02:02:15,</b> <b>C*04:175</b>
	185 bp		*31:72	
<b>27<sup>5</sup></b>	85 bp	<b>800 bp</b>	*31:31, 31:67-31:68	*02:41, 02:80, 02:117, 02:289:01, 02:304, 02:454, 23:45, 24:62, 26:10, 32:28, 32:66, 33:32:01 *02:72, 02:275
	500 bp			
<b>28<sup>5</sup></b>	110 bp	1070 bp	*31:38	*02:490N, 02:516N
	175 bp		*31:32	
	205 bp		*31:60N	
<b>29<sup>5</sup></b>	115 bp	1070 bp	*31:48	*02:140, 26:99, 33:15 *02:104
	180 bp		*31:44, 31:81	
	275 bp		*31:36	
<b>30<sup>6</sup></b>	160 bp	<b>800 bp</b>	*31:34	
	185 bp		*31:87	
	245 bp		*31:33	
<b>31<sup>5</sup></b>	75 bp	1070 bp	*31:37	*02:24:02, 02:507, 24:21:03, 24:208, 29:33, 29:51, 29:80, 32:02, 32:06, 33:07, 33:94
	120 bp		*31:41, 31:54	
<b>32<sup>9</sup></b>	<b>Negative Control</b>			

<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\*31 SSP typings.

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

Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low

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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>Due to the sharing of sequence motifs between HLA-A alleles non-HLA-A\*31 alleles will be amplified by primer mixes 1 to 19, 21 to 29 and 31.

In addition, a few HLA-B and HLA-C alleles will be amplified by primer mixes 1, 9, 25 and 26.

<sup>5</sup>HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

<sup>6</sup>Primer mixes 10, 25 and 30 may have tendencies of unspecific amplifications.

<sup>7</sup>Primer mixes 4 and 7 may weakly amplify the A\*34:01 allele.

<sup>8</sup>Primer mix 14 may give rise to a lower yield of HLA-specific PCR product than the other HLA-A\*31 primer mixes

<sup>9</sup>Primer mix 32 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. PCR product	155	80	155	165	120	130	505	155	75	115	135	245
		215			285	165		220		160	210	
Length of int. pos. control <sup>1</sup>	800	800	800	1070	800	800	1070	1070	1070	1070	1070	1070
5'-primer(s) <sup>2</sup>	127	97	423	413	97	448	317	97	413	448	362	97
	5'-ggg 3'	5'-TCA 3'	5'-gCT 3'	5'-CCg 3'	5'-TCA 3'	5'-CCT 3'	5'-gCT 3'	5'-TCA 3'	5'-CCA 3'	5'-CCT 3'	5'-gAA 3'	5'-TCA 3'
		414			445			448		706	375	
		5'-CAg 3'			5'-TCC 3'			5'-CCT 3'		5'-CgA 3'	5'-TgA 3'	
					467						445	
					5'-CTA 3'						5'-TCC 3'	
3'-primer(s) <sup>3</sup>	238	270	538	539	341	530	538	214	448	524	538	299
	5'-CCT 3'	5'-ACT 3'	5'-CAA 3'	5'-TCA 3'	5'-CgT 3'	5'-CCT 3'	5'-CAA 3'	5'-CCA 3'	5'-CAA 3'	5'-CAT 3'	5'-CAA 3'	5'-CCA 3'
	238	453			538	536		278		565		
	5'-CCT 3'	5'-TCT 3'			5'-CAA 3'	5'-ACg 3'		5'-ggC 3'		5'-CAg 3'		
	245					570		559		831		
	5'-ACg 3'					5'-CCg 3'		5'-CCg 3'		5'-TCC 3'		
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15	16	17	18	19	20	21	22	23	24
Length of spec. PCR product	85	150	150	80	160	155	110	325	75	80	80	150
		220	225	165	235	200	185		155	155	165	180
									180	190	200	220
									215			
Length of int. pos. control <sup>1</sup>	1070	800	1070	1070	1070	1070	1070	1070	1070	1070	1070	1070
5'-primer(s) <sup>2</sup>	413	448	98	98	98	413	488	302	2 <sup>nd</sup> I	97	98	97
	5'-CCA 3'	5'-CCT 3'	5'-CAC 3'	5'-CAC 3'	5'-CAC 3'	5'-CCA 3'	5'-ggT 3'	5'-ggA 3'	5'-CTC 3'	5'-TCA 3'	5'-CAC 3'	5'-TCA 3'
		629	448	769	448		635	302	98	652	652	652
		5'-CAA 3'	5'-CCT 3'	5'-Agg 3'	5'-CCT 3'		5'-gCg 3'	5'-ggA 3'	5'-CTT 3'	5'-CTg 3'	5'-CTg 3'	5'-CTg 3'
									203			
									5'-gAA 3'			
									413			
									5'-CCA 3'			
3'-primer(s) <sup>3</sup>	456	559	281	221	221	527	559	346	238	214	221	277
	5'-TCg 3'	5'-CCg 3'	5'-AgC 3'	5'-ACA 3'	5'-ACC 3'	5'-CCg 3'	5'-CgT 3'	5'-AgC 3'	5'-CCT 3'	5'-CCA 3'	5'-ACC 3'	5'-ggT 3'
		808	559	808	292	571	777		527	245	691	282
		5'-AgA 3'	5'-CCT 3'	5'-AgA 3'	5'-gTT 3'	5'-CCT 3'	5'-gCA 3'		5'-CCg 3'	5'-ACg 3'	5'-gCC 3'	5'-gAC 3'
					559					691	811	763
					5'-CCT 3'					5'-gCC 3'	5'-CAT 3'	5'-CAA 3'
										763		791
										5'-CAA 3'		5'-AgT 3'
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

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Well No.	25	26	27	28	29	30	31
Length of spec.	135	130	85	110	115	160	75
PCR product	165	185	500	175	180	185	120
	245			205	275	245	
Length of int. pos. control <sup>1</sup>	800	1070	800	1070	1070	800	1070
5'-primer(s) <sup>2</sup>	355	448	235	375	97	97	448
	5'-CCC 3'	5'-CCT 3'	5'-AgA 3'	5'-TgA 3'	5'-TCA 3'	5'-TCA 3'	5'-CCT 3'
	626	635	414	406	652	448	
	5'-CCT 3'	5'-gCg 3'	5'-CAg 3'	5'-gCT 3'	5'-CTg 3'	5'-CCT 3'	
	706			467			
	5'-CgA 3'			5'-CTA 3'			
3'-primer(s) <sup>3</sup>	448	536	290	538	238	239	482
	5'-CAA 3'	5'-ACg 3'	5'-CAA 3'	5'-CAA 3'	5'-CCC 3'	5'-gCT 3'	5'-TgC 3'
	831	539	453		331	299	524
	5'-TCC 3'	5'-TCC 3'	5'-TCT 3'		5'-CTC 3'	5'-CCC 3'	5'-CAT 3'
		777			727	568	530
		5'-gCA 3'			5'-CCA 3'	5'-CTg 3'	5'-CCT 3'
					791		
					5'-AgT 3'		
Well No.	25	26	27	28	29	30	31

<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 decreases.

<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](http://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](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

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HLA-A*31 SSP subtyping kit <sup>2</sup>																				
				Prod No.:	Well															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
					201439101	201439102	201439103	201439104	201439105	201439106	201439107	201439108	201439109	201439110	201439111	201439112	201439113	201439114	201439115	201439116
	IHWC cell line <sup>1</sup>	A*	A*																	
1	9001 SA	*24:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324	*01:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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 LKT3	*24:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT	*29:02			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*31:01			+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
15	9075 DKB	*24:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*29:02			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540	*03:01	*80:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*33:03	*74:01		-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
19	9038 BM16	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*02:17			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*02:01	*34:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*32:01			-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
25	9049 IBW9	*33:01			-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
26	9285 WT49	*02:05			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*24:10	*29:01		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB	*02:01	*29:02		-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*29:02			-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*30:01	*68:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*30:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*31:01			+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
34	9104 DHIF	*31:01			+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
35	9302 SSTO	*32:01			-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
36	9024 KT17	*02:06	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*02:17			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML	*01:01	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*02:07	*30:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*02:07			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*02:06	*02:07		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*66:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*23:01	*24:02		-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
46	9013 SCHU	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*02:16	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND	*02:01	*11:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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<b>HLA-A*31 SSP subtyping kit<sup>2</sup></b>																		
			Prod. No.:	Well														
				17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
				201439117	201439118	201439119	201439120	201560121	201439122	201439123	201560124	201439125	201439126	201439127	201439128	201439129	201439130	201439131
IHWC cell line <sup>1</sup>	A*																	
1	9001 SA	*24:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324	*01:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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 LKT3	*24:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLG A	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*24:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540	*03:01	*80:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*33:03	*74:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*02:17		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*02:01	*34:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*32:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*33:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*02:05		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*24:10	*29:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB	*02:01	*29:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*29:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*30:01	*68:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*30:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*31:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*32:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*02:06	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*02:17		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML	*01:01	*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*02:07	*30:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*02:07		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*02:06	*02:07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*66:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*23:01	*24:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*02:16	*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND	*02:01	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

**101.430-12 – including Taq polymerase, IFU-01**  
**101.430-12u – without Taq polymerase, IFU-02**

Visit [www.olerup-ssp.com](http://www.olerup-ssp.com) for  
“Instructions for Use” (IFU)

**Lot No.: 1D7**

**Lot-specific Information**

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<sup>1</sup>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.

<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 2, 5, 6, 8, 10, 11, 13 and 15 to 31 were available.

The specificities of the primers in primer solutions 2, 5, 6, 8, 10, 16, 21, 24 to 27 and 31 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 13, 15, 17, 18, 20, 22, 23, 29 and 30 it was only possible to test the 5'-primer, the 3'-primer was not possible to test.

In primer solutions 11, 19, 28 it was only possible to test the 3'-primer, the 5'-primer was not possible to test.

In primer solution 5, 10, 16, 21 and 25 to 27 one or two 5'-primers were not possible to test, and in primer solutions 1, 6, 8, 10, 16, 21, 24, 26 and 31 one or two 3'-primers were not possible to test. Additional primers in primer solution 14 were tested by separately adding 5'-primer respectively 3'-primer.

101.430-12 – including *Taq* polymerase, IFU-01  
101.430-12u – without *Taq* polymerase, IFU-02

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Lot-specific Information

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