

101.713-24/06 – including *Taq* polymerase, IFU-01  
 101.713-24u/06u – without *Taq* polymerase, IFU-02

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 “Instructions for Use” (IFU)

**Lot No.: 17X**

**Lot-specific information**

## **Olerup SSP® DQA-DQB-DR Enhanced SSP Combi Tray**

<b>Product number:</b>	101.713-24/06 – including <i>Taq</i> pol. 101.713-24u/06u – without <i>Taq</i> pol.
<b>Lot number:</b>	17X
<b>Expiry date:</b>	2017-April-01
<b>Number of tests:</b>	24 tests – Product No. 101.713-24/24u 6 tests – Product No. 101.713-06/06u
<b>Number of wells per test:</b>	95 +1
<b>Storage - pre-aliquoted primers:</b>	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. 17X.**

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

#### **The new extended Class II tray will feature:**

- High resolution DQA1 alleles.
- DRB1, DRB345, and DQB1 low resolution typing with the following enhancements:
  - Resolution of the DRB4\*01:03N and DRB5\*01:08N null alleles.
  - Resolution of the DRB3\*01, DRB3\*02 DRB3\*03 alleles, i.e. the common DR52 groups.
  - Resolution of the DRB5\*01 and DRB5\*02 alleles, i.e. the common DR51 groups.
  - Enhanced DRB1 resolution of common DRB1\*04 alleles: DRB1\*04:01, 04:02, 04:03, 04:05 and 04:07 alleles.
  - Enhanced DRB1 resolution of DRB1\*14 alleles: DRB1\*14:01 and 14:54.
  - Enhanced DQB1 resolution of common DQB1\*06 alleles: DQB1\*06:01, 06:02, 06:03, 06:04 and 06:09.

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 kit design is based on IMGT/HLA database 3.16.0.

Change in revision R01 compared to R00:

1. Primer mix 53 does not amplify the DRB1\*03:11:01, \*03:97, \*11:53 and \*15:100 alleles. This has been corrected in the Specificity and Interpretation Tables.

Change in revision R02 compared to R01:

1. Primer mix 69 does not amplify the DRB1\*08:14 allele. This has been corrected in the Specificity and Interpretation Tables.

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Well **96** 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> 5'-CAC <sup>3'</sup>	<b>340</b> 5'-Agg <sup>3'</sup>	<b>440</b> 5'-TTA <sup>3'</sup>	<b>45</b> 5'-Tgg <sup>3'</sup>	<b>45</b> 5'-Tgg <sup>3'</sup>	<b>43</b> 5'-Tgg <sup>3'</sup>	<b>36</b> 5'-TAC <sup>3'</sup>
							<b>36</b> 5'-TAT <sup>3'</sup>
<b>3'-primer<sup>2</sup></b>	<b>231</b> 5'-TgC <sup>3'</sup>	<b>2<sup>nd</sup> I</b> 5'-AAA <sup>3'</sup>	<b>507</b> 5'-TTg <sup>3'</sup>	<b>59</b> 5'-CTC <sup>3'</sup>	<b>58</b> 5'-ggC <sup>3'</sup>	<b>57</b> 5'-CTC <sup>3'</sup>	<b>47</b> 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>C*</b>	+	+	+				
<b>DRB1</b>				+	+		
<b>DRB3</b>					+	+	
<b>DRB5</b>					+		
<b>DQB1</b>						+	
<b>DPB1</b>							+
<b>DQA1</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 codonnumbering 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

### DQA-DQB-DR Enhanced SSP Combi Tray

#### CONTENT

- The primer set contains 5'- and 3'-primers for identifying the DQA1\*01:01 to DQA1\*06:02 alleles.
- The primer set contains 5'- and 3'-primers for grouping the DQB1 alleles into the serological groups DQ2 to DQ9 as well as 5'- and 3'-primers for enhanced resolution of the DQB1\*06:01, DQB1\*06:02, DQB1\*06:03, DQB1\*06:04 and DQB1\*06:09 alleles.
- The primer set contains 5'- and 3'-primers for grouping the DRB1\*01:01 to DRB1\*10:07 alleles into the corresponding serological groups DR1 to DR18, 5'- and 3'-primers for the resolution of the DRB4\*01:03N and DRB5\*01:08N null alleles, enhanced resolution of common DR52 groups DRB3\*01, DRB3\*02 and DRB3\*03 alleles, enhanced resolution of the DRB5\*01 and DRB5\*02 alleles, enhanced resolution of the DRB1\*04:01, 04:02, 04:03, 04:05 and 04:07 alleles as well as resolution of the DRB1\*14:01 and 14:54 alleles.

*Please note that DQB1 amplifications usually are somewhat less pronounced than e.g. DRB and DQA1 amplifications even when using the same DNA preparation and exactly the same experimental procedures.*

#### PLATE LAYOUT

Each test consists of 96 PCR reactions in a 96 well PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	32
33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48
49	50	51	52	53	54	55	56
57	58	59	60	61	62	63	64
65	66	67	68	69	70	71	72
73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88
89	90	91	92	93	94	95	NC

The 96 well PCR plate is marked with ‘DQA-DQB-DR’ in silver/gray ink.

Well No. 1 is marked with the Lot No. ‘17X’.

Wells 1 to 31 – DQA1 high resolution primers.

Wells 32 to 48 –DQB1 resolution primers.

Wells 49 to 95 – DRB resolution primers.

Well 96 – Negative Control.

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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.

#### INTERPRETATION

Only DQA1 alleles will be amplified by the 31 wells of the DQA1 resolution primer set, **wells 1 to 31**. Thus, the interpretation of DQA1 typings is not influenced by the DQA2 gene.

For further details see Specificity Table.

Only the DQB1 alleles will be amplified by the 17 wells of the DQB1 resolution primer set, **wells 32 to 48**. Thus, the interpretation of DQB1 resolution typings is not influenced by the DQB2 and DQB3 genes.

For further details see Specificity Table.

Only DRB alleles will be amplified by the 47 wells the DRB resolution primer set, **wells 49 to 95**. Thus, the interpretation of DRB resolution typings is not influenced by other HLA class II genes.

#### UNIQUELY IDENTIFIED ALLELES

All the DQA1 alleles, i.e. **DQA1\*01:01 to 01:12, DQA1\*02:01, DQA1\*03:01 to 03:03, DQA1\*04:01 to DQA1\*04:04, DQA1\*05:01 to 05:11 and DQA1\*06:01 to DQA1\*06:02**, recognized by the HLA Nomenclature Committee in April 2014<sup>1,2</sup> will give rise to unique amplification patterns by the primers in the DQA1 primer set, **wells 1 to 31**. The DQA1 typing kit cannot distinguish the DQA1\*01:01:01-01:01:02 alleles, the DQA1\*01:02:01:01-01:02:03 alleles, the DQA1\*01:03:01:01-01:03:01:02 alleles, the DQA1\*01:04:01:01-01:04:02 alleles, the DQA1 \*01:05:01-01:05:02, the DQA1\*03:03:01-03:03:02 alleles, the DQA1\*04:01:02-01-04:01:02:02, the DQA1\*05:01:01-01-05:01:02 alleles, the DQA1\*05:05:01:01-05:05:01:03 alleles and the DQA1\*06:01:01-06:01:02 alleles.

All the DQB1 alleles, i.e. **DQB1\*05:01:01:01 to 05:67, DQB1\*06:01:01 to 06:148, DQB1\*02:01:01 to 02:39, DQB1\*03:01:01:01 to 03:138 and DQB1\*04:01:01 to 04:22**, recognized by the HLA Nomenclature Committee in April 2014<sup>1,2</sup> will be amplified by the primers in the DQB1 primer set, **wells 32 to 48**. The DQB1 alleles will be grouped into their corresponding serological specificities<sup>4</sup>, i.e.:

DQ5(1) =	DQB1*05:01:01-05:05
DQ6(1) =	DQB1*06:01:01-06:44
DQ2 =	DQB1*02:01:01-02:05
DQ3 =	DQB1*03:06, 03:10, 03:14
DQ7(3) =	DQB1*03:01:01-03:01:06, 03:04, 03:09, 03:13, 03:16, 03:19
DQ8(3) =	DQB1*03:02:01-03:02:05, 03:05:01-03:05:04, 03:07-03:08, 03:11, 03:18
DQ9(3) =	DQB1*03:03:02:01-03:03:04, 03:12, 03:15, 03:17, 03:20
DQ4 =	DQB1*04:01-04:02

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All the DRB1, DRB3, DRB4 and DRB5 alleles, i.e. DRB1\*01:01:01 to 10:07, DRB3\*01:01:02:01 to DRB3\*03:03, DRB4\*01:01:01:01 to DRB4\*03:01N, DRB5\*01:01:01 to DRB5\*02:06, recognized by the HLA Nomenclature Committee in April 2014<sup>1,2</sup> will be amplified by the primers in the DRB low resolution SSP primer set, **wells 49 to 95**. The DRB alleles will be grouped into their corresponding serological specificities<sup>3</sup>.

<sup>1</sup>DQA1, DQB1 and DRB1, DRB3, DRB4 and DRB5 alleles listed on the IMGT/HLA web page 2014-April-14, release 3.16.0, [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 DQA1, DQB1 and the DRB1, DRB3, DRB4 and DRB5 alleles will be grouped into their corresponding serological specificities, except that following alleles give rise to identical amplification patterns:

**Alleles**

DRB1\*03:11:01, 13:02:02

DRB1\*03:76, 13:176

These alleles may be separated by the respective DRB1 high resolution kits.

<sup>4</sup>The serological split of the DQB1\*05:05 to 05:47, DQB1\*06:06 to 06:07, 06:10, 06:13, 06:15 to 06:24 and 06:27 to 06:119, the DQB1\*02:04 to 02:39 the DQB1\*03:02:02 to 03:02:04, 03:03:03, 03:05:02, 03:07 to 03:09 and 03:11 to 03:138 and the DQB1\*04:0301 to 04:22 alleles is not known. The grouping of not serologically defined alleles is taken from the expert-assigned serological grouping in Tissue Antigens (2009) 73:95-170.

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**SPECIFICITY TABLE**

**DQA1 high resolution primer set**

**Specificities and sizes of the PCR products of the 31 primer mixes used for DQA1 SSP typing**

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified DQA1 alleles <sup>3</sup>
<b>1</b>	145 bp	<b>515 bp</b>	*01:01:01-01:01:02, 01:04:01:01-01:05:02, 01:07, 01:12
<b>2</b>	170 bp	<b>515 bp</b>	*01:01:01-01:02:04, 01:04:01:01-01:09, 01:11-01:12
<b>3<sup>6</sup></b>	145 bp	430 bp	*01:02:01:01-01:03:01:02, 01:06, 01:08-01:11
<b>4</b>	170 bp	430 bp	*01:03:01:01-01:03:01:02, 01:10
<b>5</b>	220 bp	430 bp	*01:04:01:01-01:05:02, 01:06 <sup>?</sup> , 01:07, 01:08 <sup>?</sup> -01:09 <sup>?</sup> , 01:12 <sup>?</sup>
<b>6<sup>4</sup></b>	100 bp	430 bp	*01:04:01:01-01:04:02, 01:06 <sup>?</sup> , 01:07, 01:08 <sup>?</sup> -01:09 <sup>?</sup> , 01:12 <sup>?</sup>
<b>7<sup>4</sup></b>	95 bp	430 bp	*01:06
<b>8<sup>4</sup></b>	65 bp	430 bp	*01:01:01-01:02:03, 01:03:01:01-01:03:01:02, 01:06 <sup>?</sup> , 01:08 <sup>?</sup> -01:09 <sup>?</sup> , 01:10-01:11, 01:12 <sup>?</sup> , 02:01, 03:01:01-03:03:02, 04:01:01-04:04, 05:01:01:01-05:11, 06:01:01-06:02
<b>9</b>	175 bp	430 bp	*02:01
<b>10</b>	185 bp	430 bp	*03:01:01-03:03:02
<b>11</b>	215 bp	430 bp	*03:02
<b>12</b>	225 bp	<b>515 bp</b>	*03:02-03:03:02
<b>13</b>	225 bp	<b>515 bp</b>	*01:01:01-01:12, 02:01, 03:01:01, 04:01:01, 04:02-04:04, 05:01:01-05:11, 06:01:01-06:02
<b>14<sup>4,6</sup></b>	125 bp	430 bp	*04:01:01-04:02, 04:04, 05:01:01-05:11
<b>15</b>	165 bp	430 bp	*05:01:01-01-05:09, 05:11
<b>16<sup>4</sup></b>	95 bp	<b>515 bp</b>	*05:02, 05:07
<b>17</b>	200 bp	430 bp	*05:01:01:01-05:01:02, 05:02 <sup>?</sup> , 05:04 <sup>?</sup> , 05:05:01:01-05:05:01:03, 05:08-05:11
<b>18</b>	200 bp	430 bp	*05:02 <sup>?</sup> , 05:03, 05:04 <sup>?</sup> , 05:06-05:07
<b>19</b>	205 bp	430 bp	*05:01:01:01-05:03, 05:05:01:01-05:09, 05:11
<b>20</b>	135 bp	430 bp	*05:04
<b>21<sup>4</sup></b>	100 bp, 210 bp	430 bp	*01:09, 05:02 <sup>?</sup> , 05:04 <sup>?</sup> , 05:05:01:01-05:05:01:03, 05:08-05:09, 05:10 <sup>?</sup> , 05:11
<b>22<sup>4,5</sup></b>	120 bp, 215 bp	<b>515 bp</b>	*01:10, 06:01:01-06:02
<b>23<sup>4</sup></b>	85 bp	430 bp	*04:01:01-04:04, 06:01:01-06:02
<b>24</b>	220 bp	430 bp	*01:01:01-01:12, 02:01 <sup>w</sup> , 03:01:01-03:03:02, 04:01:01-04:04, 05:01:01-05:01:02, 05:02 <sup>?</sup> , 05:03, 05:04 <sup>?</sup> , 05:06-05:07, 05:10 <sup>?</sup> , 06:01:01-06:02

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<b>25<sup>4</sup></b>	80 bp, 175 bp	430 bp	*01:07, 05:09
<b>26</b>	160 bp	430 bp	*04:02, 05:10
<b>27<sup>4,6</sup></b>	90 bp , 135 bp	430 bp	*01:11, 04:03N
<b>28<sup>4</sup></b>	105 bp	430 bp	*04:04
<b>29</b>	150 bp, 250 bp	430 bp	*01:08, 01:12, 06:02
<b>30<sup>4</sup></b>	115 bp, 215 bp	430 bp	*05:06, 05:11
<b>31</b>	135 bp	430 bp	*05:08

<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 DQA1 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.

<sup>2</sup>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.

<sup>3</sup>For several DQB1 alleles 1<sup>st</sup> and/or 3<sup>rd</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>Specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

<sup>5</sup>Primer mix 22 may have tendencies of unspecific amplifications.

<sup>6</sup>Primer mixes 3 and 14 may give rise to a lower yield of HLA-specific PCR product than the other DQA1 primer mixes.

‘w’, may be weakly amplified.

‘?’, nucleotide sequence information not available for the primer matching sequence.

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**SPECIFICITY TABLE**

**DQB1 resolution primer set**

**Specificities and sizes of the PCR products of the 17 primer mixes of the DQB1 resolution primer set**

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	DQ serology <sup>3</sup>	Amplified DQB1 alleles <sup>4</sup>
32	135 bp, 230 bp	<b>515 bp</b>	5	*05:01:01:01-05:59, 05:61-05:67
33	140 bp, 185 bp, 220 bp, 270 bp	<b>515 bp</b>	1, 5, 6	*06:01:01-06:145, 06:147-06:148
34	210 bp	430 bp	2	*02:01:01-02:39
35	220 bp	<b>515 bp</b>	3, 7	*03:01:01:01-03:01:24, 03:04:01-03:04:02, 03:09-03:10:02, 03:13-03:14:02, 03:16, 03:19, 03:21-03:22, 03:24, 03:27-03:29, 03:35-03:36, 03:42, 03:44, 03:46-03:60, 03:69, 03:71, 03:73, 03:75-03:77, 03:80, 03:82-03:84N, 03:92-03:94, 03:101-03:103, 03:108, 03:114-03:116, 03:118N-03:122, 03:127-03:131, 03:133-03:135, 03:138
36 <sup>5</sup>	130 bp, 220 bp	<b>515 bp</b>	6, 8	*03:02:01-03:02:14, 03:05:01-03:05:04, 03:07-03:08, 03:11, 03:18, 03:32, 03:37, 03:45, 03:61, 03:63-03:64, 03:66N-03:68, 03:70, 03:85, 03:104, 03:106-03:107, 03:125, 03:132, 06:29, 06:123, 06:139
37 <sup>5,7</sup>	135 bp	<b>515 bp</b>	2, 3, 6, 9	*02:03, 03:03:02:01-03:03:12, 03:06, 03:12, 03:15, 03:20, 03:25-03:26, 03:30-03:31, 03:33-03:34, 03:38-03:41, 03:43, 03:65, 03:74, 03:79, 03:86-03:91Q, 03:95N- 03:99Q, 03:104-03:105, 03:111-03:113, 03:117, 03:123-03:124, 03:126, 03:136- 03:137, 04:03:01-04:03:02, 06:03:10, 06:51:01, 06:66, 06:96
38 <sup>5,6,7</sup>	85 bp	<b>515 bp</b>	3, 7, 8, 9	*03:01:01:01-03:01:06, 03:01:08-03:05:04, 03:07, 03:09-03:24, 03:26-03:57, 03:59- 03:64, 03:66N-03:103, 03:105-03:106, 03:108-03:136, 03:138
39	160 bp, 205 bp	430 bp	4	*03:132, 04:01:01-04:22
40	170 bp	430 bp	4, 5	*04:01:01-04:02:06, 04:04-04:19, 04:21, 04:22 <sup>w</sup> , 05:03:02 <sup>2</sup> , 05:04, 05:52
41	215 bp	430 bp	5	*05:01:01:01-05:03:09, 05:03:11-05:03:13, 05:05:01-05:33, 05:35-05:43, 05:45-05:51, 05:53, 05:55-05:67
42	185 bp	430 bp	8, 9	*03:03:11, 03:05:01, 03:05:03, 03:17:01, 03:61, 03:72, 03:100
43 <sup>7</sup>	185 bp	430 bp	4, 7, 8, 9	*03:01:01:01-03:01:01:03, 03:01:03- 03:01:07, 03:01:09-03:02:02, 03:02:04- 03:02:12, 03:02:14-03:03:02:03, 03:03:04- 03:04:02, 03:05:03-03:17:01, 03:18-03:19, 03:21-03:22, 03:24-03:36, 03:38-03:60,

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					03:62-03:71, 03:74, 03:76-03:98, 03:101-03:103, 03:106-03:108, 03:110- 03:111, 03:113-03:117, 03:119-03:131, 03:133-03:138, 04:01:03
44	220 bp	<b>515 bp</b>	6		*06:01:01-06:01:12, 06:35, 06:43, 06:45, 06:53-06:57, 06:82, 06:98-06:105, 06:108, 06:120, 06:132, 06:140
45	190 bp	430 bp	6		*04:10, 06:02:01-06:02:17, 06:14:01-06:16, 06:19:01-06:20, 06:23-06:24, 06:33, 06:37, 06:46-06:50, 06:51:02, 06:68, 06:70-06:84, 06:95, 06:97, 06:107, 06:109, 06:111- 06:117, 06:122, 06:124-06:127, 06:136- 06:138, 06:146-06:147
46	160 bp, 210 bp	<b>515 bp</b>	6		*06:03:01-06:03:15, 06:08:01-06:08:02, 06:14:01-06:14:02, 06:21, 06:27:01-06:28, 06:30-06:32, 06:40-06:41, 06:44, 06:59- 06:65, 06:67, 06:87, 06:90-06:92, 06:110, 06:128, 06:133-06:134, 06:141, 06:143- 06:145, 06:148
47	210 bp	<b>515 bp</b>	6		*06:04:01-06:04:08, 06:07:01-06:07:02, 06:17, 06:21, 06:25, 06:34, 06:36, 06:38- 06:39, 06:52, 06:58, 06:69, 06:85-06:86, 06:89, 06:92-06:93, 06:135
48	210 bp	430 bp	5, 6		*06:05:01, 06:05:02 <sup>2</sup> -06:06 <sup>2</sup> , 06:09:01- 06:09:05, 06:12, 06:15:01-06:15:02, 06:22:01-06:22:02, 06:42, 06:46, 06:66, 06:88, 06:94, 06:118:01-06:119, 06:121, 06:142

<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 DQB1 resolution 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.

<sup>2</sup>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 32 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. In the presence of a specific amplification the intensity of the control band often decreases.

<sup>3</sup>The serological split of the DQB1\*05:05 to 05:47, DQB1\*06:06 to 06:07, 06:10, 06:13, 06:15 to 06:24 and 06:27 to 06:119, the DQB1\*02:04 to 02:39 the DQB1\*03:02:02 to 03:02:04, 03:03:03, 03:05:02, 03:07 to 03:09 and 03:11 to 03:138 and the DQB1\*04:0301 to 04:22 alleles is not

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known. In this table we have used the expert-assigned serological grouping in Tissue Antigens (2009) 73:95-170, and also inferred the serological grouping from the naming of the sequence-defined allele.

<sup>4</sup>For several DQB1 alleles 1<sup>st</sup> and/or 3<sup>rd</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>5</sup>Specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

<sup>6</sup>The primer pairs in well 38 will in some samples give rise to two HLA-specific PCR fragments.

<sup>7</sup>Primer mixes 38 and 43 may give a lower yield of HLA-specific PCR products than the other DQB1 primer mixes.

'ser', serological HLA specificity

'w', may be weakly amplified.

'?', nucleotide sequence information not available for the primer matching sequence.

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**SPECIFICITY TABLE**

**DRB resolution primer set**

**Specificities and sizes of the PCR products of the 47 primer mixes used for DRB resolution primer set**

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	DR serology <sup>3</sup>	Amplified HLA-DRB alleles <sup>4,6</sup>
49 <sup>6,7</sup>	210 bp, 235 bp, 260 bp	515 bp	1	*01:01:01-01:02:09, 01:04-01:38, 01:40N-01:62N
50	200 bp	430 bp	1/103	*01:03, 01:39N, 01:42, 01:61
51 <sup>6,8</sup>	210 bp, 230 bp	430 bp	2, 15	*15:01:01:01-15:107
52 <sup>8</sup>	210 bp	430 bp	16	*16:01:01-16:05:02, 16:07-16:24
53 <sup>5,6,10</sup>	120 bp, 220 bp	430 bp	3, 11, 17, 18	*03:01:01:01-03:10, 03:12-03:75, 03:77-03:96, 03:98-03:103, 11:07, 11:103, 11:105, 11:107, 11:125, 15:25
54 <sup>5,6,10,12</sup>	75 bp, 210 bp	430 bp	3, 6, 11, 13, 14, 17	*03:01:01:01-03:01:22, 03:04:01-03:06, 03:08-03:16, 03:18-03:20, 03:22-03:23, 03:25-03:26, 03:28, 03:30-03:31, 03:33-03:34, 03:36-03:37, 03:43-03:48, 03:50-03:52, 03:54-03:68N, 03:70-03:73, 03:75-03:86, 03:89, 03:91-03:93, 03:95-03:96, 03:98-03:100:02, 08:40, 11:02:01-11:03, 11:11:01-11:11:03, 11:14:01-11:14:02, 11:16, 11:20-11:21, 11:36, 11:40-11:41, 11:48, 11:59, 11:63:01, 11:65:01-11:65:02, 11:68, 11:70, 11:73, 11:76, 11:79-11:80, 11:83, 11:85-11:87, 11:93, 11:118, 11:122, 11:124, 11:127, 11:131-11:132, 11:135, 11:138-11:139, 11:142, 11:151, 11:153, 13:01:01-13:04, 13:08, 13:10, 13:15-13:17, 13:19-13:20, 13:22-13:24, 13:27-13:29, 13:31-13:41, 13:43, 13:45, 13:48, 13:51-13:54, 13:57, 13:59, 13:61:01-13:61:02, 13:63-13:66:02, 13:68-13:76, 13:78-13:81, 13:83-13:85, 13:87-13:99, 13:101-13:102, 13:104-13:107, 13:109, 13:111-13:117, 13:120-13:131, 13:133, 13:135, 13:137N-13:145, 13:147-13:149, 13:151-13:153, 13:155, 13:159, 13:162, 13:165-13:168, 13:170-13:179, 14:16, 14:19, 14:21, 14:82, 14:95, 14:109, 14:132, 14:137N
55 <sup>5,6</sup>	85 bp, 210 bp	430 bp	3, 6, 11, 13, 14, 1403, 18	*03:02:01-03:03, 03:27, 03:29, 03:38, 03:53, 03:74, 03:88, 03:90, 03:102-03:103, 11:13:01 <sup>w</sup> -11:13:02 <sup>w</sup> , 11:26, 11:34, 13:15, 13:19, 13:26:01-13:26:02, 13:44, 13:53, 13:57, 13:85-13:86, 13:104, 14:02:01-14:03:02, 14:06:01-14:06:03, 14:09, 14:12:01-14:13, 14:17-14:21, 14:24, 14:27, 14:29-14:30, 14:32:01 <sup>w</sup> -14:32:02 <sup>w</sup> , 14:33, 14:40-14:41, 14:47-14:49, 14:51, 14:63, 14:65 <sup>w</sup> , 14:67, 14:77-14:78, 14:80-14:81, 14:83, 14:85, 14:89, 14:94, 14:98, 14:102, 14:106, 14:108-14:109, 14:115, 14:119, 14:121, 14:135, 14:146
56 <sup>5,6</sup>	100 bp, 175 bp	430 bp	3, 4	*04:01:01-04:05:11, 04:05:13-04:176
57	235 bp	430 bp	7	*07:01:01:01-07:01:07, 07:03-07:29

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<b>58<sup>6</sup></b>	170 bp, 215 bp, 250 bp	<b>515 bp</b>	8, 11, 12, 14	*08:01:01-08:19, 08:21-08:56, 11:67, 12:04, 12:16:01-12:16:03, 12:22, 12:39, 14:11, 14:15, 14:68, 14:93, 14:148
<b>59<sup>5,6</sup></b>	90 bp, 135 bp, 190 bp	<b>430 bp</b>	3, 9, 11	*03:08, 03:65, 09:01:02-09:22, 11:07, 11:53, 11:103, 11:105, 11:107, 11:125
<b>60</b>	180 bp	<b>430 bp</b>	10, 11, 13	*03:76, 10:01:01-10:07, 11:59, 11:80, 11:83, 11:87, 11:135, 11:142, 13:27, 13:41, 13:71, 13:129, 13:176
<b>61<sup>5,6</sup></b>	100 bp, 170 bp	<b>430 bp</b>	3, 8, 11, 13, 14	*03:08, 03:65, 08:31, 08:41, 11:01:01-11:70, 11:72-11:158
<b>62<sup>5,6</sup></b>	90 bp, 110 bp	<b>430 bp</b>	12	*08:32, 08:53, 12:01:01-12:46
<b>63</b>	220 bp	<b>430 bp</b>	6, 8, 11, 13, 14, 1403	*03:76, 08:20-08:21, 11:01:01-11:04:11, 11:06:01-11:06:03, 11:08:01-11:12:02, 11:14:01-11:16, 11:18-11:21, 11:23:01-11:25, 11:27:01-11:33, 11:35-11:51, 11:54:01-11:54:02, 11:56-11:66, 11:68, 11:70, 11:72-11:81, 11:83-11:88, 11:90-11:97, 11:99-11:102:02, 11:106, 11:108-11:124, 11:126-11:135, 11:137-11:142, 11:144-11:158, 13:01:01-13:02:01, 13:02:03-13:08, 13:10-13:16, 13:18-13:43, 13:45-13:85, 13:87-13:115, 13:117-13:128, 13:130-13:145, 13:147-13:166, 13:168-13:174, 13:176-13:179, 14:03:01-14:03:02, 14:12:01-14:12:02, 14:16, 14:19, 14:21-14:22, 14:25, 14:27, 14:40, 14:53, 14:63, 14:67, 14:69, 14:74, 14:77-14:78, 14:84-14:85, 14:98, 14:102, 14:105, 14:109, 14:115-14:116, 14:128, 14:135, 14:137N, 14:144, DRB3*02:27
<b>64<sup>6</sup></b>	200 bp, 225 bp	<b>430 bp</b>	6, 8, 11, 12, 13, 14	*08:01:01-08:01:05, 08:02:01-08:02:04, 08:04:01-08:09, 08:11, 08:16-08:17, 08:20-08:22, 08:24, 08:26, 08:28, 08:31, 08:39, 08:41-08:44, 08:50, 08:52, 08:54-08:55, 11:01:01-11:01:17, 11:01:20-11:06:03, 11:09-11:12:02, 11:14:01-11:16, 11:20-11:21, 11:23:01-11:25, 11:27:01-11:30, 11:32-11:33, 11:35-11:41, 11:43-11:44, 11:46:01-11:51, 11:54:01-11:56, 11:58:01-11:63:01, 11:65:01-11:70, 11:72, 11:74:01-11:78, 11:80-11:88, 11:90-11:97, 11:99-11:102:02, 11:106, 11:108-11:118, 11:120-11:124, 11:126-11:129, 11:133-11:135, 11:137-11:142, 11:144-11:152, 11:154-11:158, 12:02:01-12:02:06, 12:13, 12:15-12:16:03, 12:18-12:21, 12:23, 12:26-12:27, 12:31N-12:33, 12:37, 12:42-12:45, 13:01:01-13:02:01, 13:02:03-13:02:09, 13:04-13:05:02, 13:07:01-13:09, 13:11:01-13:11:02, 13:14:01-13:24, 13:26:01-13:29, 13:31-13:32, 13:34-13:36, 13:38-13:43, 13:45-13:55, 13:57, 13:59, 13:61:01-13:65, 13:67-13:76, 13:78-13:80, 13:83-13:84, 13:87, 13:91-13:93, 13:96:01-13:100, 13:102-13:109, 13:111-13:114, 13:116-13:117, 13:121, 13:123-13:132, 13:135-13:136, 13:138-13:150, 13:153, 13:155, 13:158-13:160, 13:162, 13:164-13:166, 13:168-13:169, 13:171, 13:173, 13:175, 13:177, 13:179, 14:15-14:16, 14:22, 14:24-14:25, 14:27, 14:37, 14:53, 14:73, 14:105, 14:128
<b>65<sup>10</sup></b>	175 bp	<b>430 bp</b>	3, 6, 11, 13, 14, 1403, 17, 18	*03:01:01:01-03:07, 03:09, 03:11:01-03:41, 03:43-03:45, 03:47-03:63, 03:66-03:68N, 03:70-03:86, 03:88-03:91, 03:93-03:103, 08:20, 13:01:01-13:16, 13:18-13:42, 13:44, 13:46-13:66:02, 13:68-13:102, 13:104-13:115, 13:117-13:121, 13:123-13:158, 13:161-13:164, 13:166-13:170, 13:172-13:174, 13:176-13:178,

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66 <sup>5,6,11</sup>	100 bp, 150 bp, 195 bp, 240 bp	430 bp	4, 6, 8, 13, 14, 1404	*04:62, 04:69, 04:73, 04:105:01-04:105:02, 04:122, 04:146, 08:08, 11:69, 11:82, 13:45, 14:01:01-14:01:02, 14:01:04, 14:04, 14:07:01-14:07:02, 14:10, 14:16, 14:22, 14:25-14:26, 14:28, 14:31-14:32:03, 14:35, 14:37-14:39, 14:49-14:50, 14:53-14:54:01, 14:54:03-14:55, 14:57-14:58, 14:60-14:62, 14:68-14:71, 14:73-14:76, 14:79, 14:82, 14:86-14:88, 14:90, 14:93, 14:99, 14:101, 14:104-14:105, 14:107, 14:110-14:114, 14:117-14:120, 14:122, 14:124-14:125, 14:128-14:129, 14:131, 14:137N-14:140, 14:142-14:143, 14:145-14:147, 14:149-14:150, <b>DRB4*01:03:01:02N</b>
67 <sup>5,6,7,9</sup>	110 bp, 145 bp, 170 bp	430 bp	3, 4, 6, 9, 11, 13, 14, 1404	*03:10, 09:01:02-09:01:05, 09:01:07-09:02:02, 09:04-09:22, 11:13:01-11:13:02, 11:17, 11:52, 13:43, 13:159, 13:171, 13:179, 14:01:01-14:02:02, 14:04-14:11, 14:13-14:14, 14:16-14:18, 14:19 <sup>w</sup> , 14:20, 14:21 <sup>w</sup> , 14:22-14:23:04, 14:26, 14:28-14:36, 14:38-14:39, 14:41, 14:43-14:52, 14:54:01-14:57, 14:59-14:62, 14:64-14:65, 14:68, 14:70-14:76, 14:79-14:83, 14:86-14:88, 14:90-14:97, 14:99-14:101, 14:103-14:108, 14:109 <sup>w</sup> , 14:110-14:114, 14:117-14:127:02, 14:129-14:134, 14:137N-14:140, 14:142-14:143, 14:145-14:150, 15:27, 15:34, 15:66:01-15:66:02
68 <sup>5,6</sup>	110 bp, 150 bp, 180 bp, 220 bp	430 bp	2 <sup>w</sup> , 3, 4, 6, 8, 11, 13, 14, 1403, 1404, 16 <sup>w</sup>	*03:10, 08:09, 08:20-08:21, 08:32, 08:35, 08:36:02, 08:53, 11:13:01-11:13:02, 11:17, 11:23:01-11:23:02, 11:25, 11:31, 11:45, 11:52, 11:55, 11:64, 11:89, 11:96, 11:119, 11:148, 13:13, 13:18, 13:43, 13:45, 13:47, 13:55, 13:119, 13:144, 13:146, 13:154, 13:156, 13:158-13:159, 13:164, 13:171, 13:179, 14:01:01-14:01:04, 14:03:01-14:05:04, 14:07:01-14:08, 14:10-14:12:02, 14:14-14:16, 14:18, 14:22-14:23:04, 14:25-14:28, 14:31-14:32:03, 14:34-14:36, 14:38-14:40, 14:42-14:45, 14:49-14:50, 14:53-14:65, 14:67-14:79, 14:81-14:82, 14:84-14:93, 14:95-14:97, 14:99-14:105, 14:107, 14:110-14:120, 14:122-14:140, 14:142-14:150, 15:21 <sup>w</sup> , 16:04 <sup>w</sup> , 16:18 <sup>w</sup>
69	165 bp	430 bp	8, 12, 13, 14	*03:92, 08:01:01-08:04:05, 08:04:07-08:06, 08:09-08:10, 08:12-08:13, 08:16-08:18, 08:21-08:24, 08:26-08:30:03, 08:32-08:33, 08:35-08:40, 08:42-08:51, 08:53-08:56, 12:09, 12:46, 13:17, 13:116, 13:175, 14:15, 14:52, 14:126
70 <sup>5,6,7</sup>	75 bp, 175 bp, 265 bp	430 bp	2, 4, 8, 11, 13, 14	*04:12, 04:18, 04:25, 04:58, 08:04:01, 08:04:02 <sup>w</sup> -08:04:03 <sup>w</sup> , 08:04:04-08:04:07, 08:06, 08:10, 08:12, 08:14, 08:20, 08:22, 08:28, 08:54, 11:25, 11:67, 11:119, 13:18, 13:144, 13:156, 13:164, 14:12:01-14:12:02, 14:15, 14:78, 14:84, 15:21
71	135 bp	430 bp	8, 12, 13	*03:92, 08:17, 08:28, 08:37, 08:45, 11:67, 12:01:01-12:20, 12:22-12:37, 12:39-12:41, 12:43-12:46, 13:17, 13:175, 14:138

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72	180 bp	430 bp	3, 6, 11, 13, 14	*03:12, 13:03:01-13:04, 13:12:01-13:13, 13:21:01- 13:21:02, 13:30, 13:32-13:33:03, 13:38, 13:48-13:49, 13:55, 13:58, 13:65-13:66:02, 13:75, 13:81, 13:88-13:90, 13:93-13:95, 13:101, 13:108, 13:115, 13:118, 13:120, 13:122, 13:133-13:134, 13:139, 13:151-13:152, 13:154, 13:161, 13:164, 13:167, 13:169, 13:174, 14:13, 14:63, 14:65, 14:78, 14:85, <b>DRB5*01:08N</b>
73	150 bp	430 bp	2, 4, 6, 11, 13	*11:16, 11:20, 11:40, 11:59, 11:118, 11:122, 13:01:01- 13:02:01, 13:02:03-13:02:09, 13:08, 13:15-13:16, 13:19- 13:20, 13:27-13:29, 13:31-13:32, 13:34-13:36, 13:39- 13:41, 13:43, 13:51-13:53, 13:57, 13:59, 13:61:01- 13:61:02, 13:63-13:65, 13:67-13:69, 13:71-13:74, 13:76, 13:78-13:80, 13:83-13:84, 13:87, 13:91-13:93, 13:96:01- 13:97:01, 13:98-13:99, 13:102-13:107, 13:109, 13:111- 13:114, 13:117, 13:121, 13:123-13:128, 13:130-13:131, 13:135, 13:138-13:143, 13:145, 13:147-13:148, 13:153, 13:155, 13:159-13:160, 13:165-13:166, 13:168, 13:171, 13:173, 13:175, 13:177, 13:179, 14:16, 14:57, 15:10
74	145 bp	430 bp	3, 4, 6, 11, 13, 14, 17	*03:01:01:01-03:01:22, 03:04:01-03:05:03, 03:07-03:16, 03:18-03:25, 03:28-03:30, 03:32-03:34, 03:36-03:37, 03:39-03:40, 03:43-03:52, 03:54-03:59, 03:61-03:68N, 03:70-03:73, 03:76-03:79, 03:81, 03:83-03:84, 03:86, 03:89, 03:91, 03:93-03:101, 11:01:01-11:01:06, 11:01:08-11:04:11, 11:06:01-11:16, 11:18-11:21, 11:23:01-11:29, 11:31-11:36, 11:38-11:40, 11:42-11:47, 11:49:01-11:49:02, 11:51, 11:53-11:55, 11:57-11:66, 11:68-11:70, 11:72-11:82, 11:84:01-11:87, 11:89-11:103, 11:105-11:106, 11:108-11:144, 11:146-11:148, 11:150- 11:158, 13:01:01-13:01:08, 13:01:10-13:02:09, 13:04- 13:06, 13:09-13:11:02, 13:14:01-13:16, 13:18, 13:20- 13:25, 13:27-13:31, 13:34, 13:39, 13:41-13:46, 13:50:01- 13:52, 13:54, 13:56-13:57, 13:59, 13:61:01-13:64, 13:66:01-13:66:02, 13:68-13:69, 13:71, 13:73-13:75, 13:77-13:80, 13:82-13:83, 13:86-13:87, 13:91-13:93, 13:96:01-13:100, 13:102, 13:104-13:107, 13:109-13:114, 13:117, 13:119, 13:121, 13:123-13:132, 13:136-13:148, 13:150, 13:153-13:158, 13:162-13:163, 13:165-13:166, 13:168-13:173, 13:176, 13:178-13:179, 14:17, 14:21, 14:30, 14:33, 14:35, 14:42, 14:53, 14:64-14:65, 14:72, 14:95, 14:128, 14:132, 14:134
75 <sup>6</sup>	145 bp, 210 bp, 235 bp	430 bp	4, 6, 8, 11, 13, 14, 1403, 1404	*08:09, 08:20-08:21, 08:35, 11:13:01-11:13:02, 11:17, 11:23:01-11:23:02, 11:25, 11:45, 11:52, 11:55, 11:64, 11:89, 11:119, 11:149, 13:08, 13:13, 13:18, 13:47, 13:55, 13:119, 13:144, 13:146, 13:154, 13:156, 13:158, 13:164, 14:01:01-14:01:04, 14:02:02 <sup>?</sup> , 14:03:01- 14:05:04, 14:07:01-14:08, 14:09 <sup>?</sup> , 14:10-14:12:02, 14:14- 14:15, 14:16 <sup>?</sup> -14:17 <sup>?</sup> , 14:18, 14:19 <sup>?</sup> -14:20 <sup>?</sup> , 14:22 <sup>?</sup> , 14:23:01-14:23:04, 14:24 <sup>?</sup> -14:25 <sup>?</sup> , 14:26-14:28, 14:29 <sup>?</sup> - 14:30 <sup>?</sup> , 14:31-14:32:02, 14:33 <sup>?</sup> , 14:34-14:36, 14:37 <sup>?</sup> , 14:38-14:40, 14:41 <sup>?</sup> , 14:42-14:45, 14:47 <sup>?</sup> -14:49 <sup>?</sup> , 14:50, 14:51 <sup>?</sup> -14:53 <sup>?</sup> , 14:54:01-14:65, 14:67-14:68, 14:69 <sup>?</sup> , 14:70-14:72, 14:73 <sup>?</sup> -14:74 <sup>?</sup> , 14:75, 14:76 <sup>?</sup> , 14:77-14:78, 14:79 <sup>?</sup> -14:80 <sup>?</sup> , 14:81-14:82, 14:83 <sup>?</sup> , 14:84-14:93, 14:94 <sup>?</sup> , 14:95-14:97, 14:98 <sup>?</sup> , 14:99-14:103, 14:104 <sup>?</sup> -14:109 <sup>?</sup> , 14:110, 14:111 <sup>?</sup> , 14:112-14:118, 14:119 <sup>?</sup> , 14:120,

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76	140 bp	430 bp	6, 13, 14	14:121 <sup>?</sup> , 14:122-14:127:02, 14:128 <sup>?</sup> , 14:129-14:145, 14:146 <sup>?</sup> , 14:147-14:150 *03:76, 13:10, 13:85, 13:120, 13:170, 13:176, 13:178, 14:02:01-14:02:02, 14:06:01-14:06:03, 14:09, 14:13, 14:17, 14:19, 14:21, 14:29-14:30, 14:33, 14:46-14:48, 14:51-14:52, 14:80, 14:83, 14:106, 14:108-14:109, 14:121
77 <sup>6</sup>	160 bp, 240 bp	430 bp	52	*14:141, DRB3*01:01:02:01-01:15, DRB3*02:01- 02:29N, DRB3*03:01:01-03:03
78 <sup>11</sup>	215 bp	430 bp	53	DRB4*01:01:01:01-01:08
79	175 bp	430 bp	51	DRB5*01:01:01-01:14, DRB5*02:02-02:06
80	215 bp	<b>515 bp</b>	4	*04:01:01-04:01:14, 04:09, 04:13, 04:16, 04:21, 04:26, 04:33, 04:35, 04:38, 04:62-04:63, 04:67, 04:72:01- 04:72:02, 04:76, 04:100, 04:111-04:115, 04:117, 04:119N, 04:123, 04:125, 04:127, 04:130, 04:135, 04:139, 04:145, 04:150-04:151, 04:153, 04:158N- 04:159, 04:171, 04:174-04:175
81	215 bp	430 bp	4, 6	*04:02:01-04:02:03, 04:14, 04:37, 04:106, 04:124, 04:132-04:134, 04:138, 04:143, 04:161, 14:57
82	225 bp	430 bp	4, 14	*04:03:01-04:03:10, 04:06:01-04:07:04, 04:11:01- 04:11:03, 04:17:01-04:17:02, 04:20, 04:27, 04:39, 04:41, 04:46, 04:49-04:52, 04:59-04:60, 04:65, 04:69, 04:71, 04:78, 04:85, 04:88, 04:91-04:93, 04:95:02-04:97, 04:100-04:102, 04:109-04:110, 04:114, 04:129, 04:136, 04:142N, 04:144, 04:146, 04:148, 04:150, 04:154, 04:156, 04:163, 04:165, 04:169-04:170, 04:172, 04:176, 14:10, 14:143
83	170 bp	430 bp	4	*04:05:01-04:05:11, 04:05:13-04:05:16, 04:09-04:11:02, 04:12, 04:17:01-04:17:02, 04:24, 04:28-04:30, 04:45, 04:48, 04:57, 04:67, 04:77, 04:80-04:81N, 04:83-04:84, 04:86-04:87, 04:89-04:91, 04:103-04:104, 04:106- 04:107, 04:116, 04:125-04:126, 04:131, 04:136-04:137, 04:147, 04:152, 04:162, 04:173
84 <sup>5</sup>	75 bp	430 bp	4, 9, 14	*01:17, 04:07:01-04:07:04, 04:17:01-04:17:02, 04:20, 04:69, 04:74, 04:92, 04:102, 04:129, 04:160, 04:170, 04:172, 09:01:02-09:01:05, 09:01:08-09:02:02, 09:04- 09:05, 09:07-09:08, 09:10-09:22, 14:07:01-14:07:02, 14:14, 14:36, 14:42, 14:44:01-14:44:03, 14:51, 14:68, 14:93, 14:134, 14:143, DRB5*01:12
85 <sup>10</sup>	250 bp	<b>515 bp</b>	14	*14:01:01-14:01:04
86	210 bp	430 bp	6, 11, 14	*11:13:01-11:13:02, 11:17, 11:52, 11:89, 14:01:01- 14:01:04, 14:05:01-14:05:04, 14:07:01-14:08, 14:14, 14:18, 14:23:01-14:23:04, 14:26, 14:32:01-14:32:02, 14:34-14:36, 14:38-14:39, 14:42-14:45, 14:54:01- 14:54:03, 14:56, 14:58-14:60, 14:62, 14:64-14:65, 14:70, 14:72, 14:75, 14:81-14:82, 14:86-14:88, 14:90-14:92N, 14:95-14:97, 14:99-14:101, 14:103, 14:110, 14:112- 14:114, 14:117-14:118, 14:122-14:125, 14:127:01- 14:127:02, 14:129-14:134, 14:139-14:140, 14:142, 14:147, 14:149-14:150
87	135 bp	430 bp	6,11, 12, 13, 14, 15, 17	*01:44:01, 03:01:17, 03:97, 11:01:05, 11:01:10, 11:04:03, 11:06:02, 11:13:01-11:13:02, 11:54:01, 12:01:01, 12:01:04-12:02:01, 12:02:03-12:02:06, 12:03:03-12:10, 12:12-12:15, 12:16:02, 12:17-12:20,

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				12:23-12:37, 12:39-12:42, 12:44-12:46, 13:01:03, 13:163, 14:35, 14:42, 14:64-14:65, 14:72, 14:95, 14:132, 14:134, 14:138, 15:01:03, 15:02:06
88	165 bp	430 bp	52	*14:141, DRB3*01:07, DRB3*02:01-02:08, DRB3*02:11-02:29N
89 <sup>5</sup>	95 bp	430 bp	14, 52	*03:42, 14:46, DRB3*01:01:02:01-01:08, DRB3*01:10- DRB3*01:13, DRB3*01:15
90 <sup>5</sup>	120 bp	430 bp	53	DRB3*02:03, DRB3*03:01:01-03:03
91	155 bp	430 bp	53	DRB4*01:03:01:02N
92	130 bp	430 bp	53	DRB4*01:01:01:01, DRB4*01:04 <sup>2</sup> -01:05 <sup>2</sup> , DRB4*01:06, DRB4*01:07 <sup>2</sup> -01:08 <sup>2</sup> , DRB4*02:01N, DRB4*03:01N
93 <sup>5</sup>	100 bp	430 bp	51	DRB5*01:01:01-01:01:02, DRB5*01:04, DRB5*01:06- 01:07, DRB5*01:09, DRB5*01:11
94	185 bp	430 bp		DRB5*02:02, DRB5*02:04-02:06
95	195 bp	430 bp		DRB5*01:08N
96 <sup>13</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 DRB resolution 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.

<sup>2</sup>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 49 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. In the presence of a specific amplification the intensity of the control band often decreases.

<sup>3</sup>The serological reactivity of all DRB alleles is not known. The grouping of not serologically defined alleles is taken from Tissue Antigens 73, 95-170, 2009.

<sup>4</sup>For several DRB1 alleles 1<sup>st</sup> and/or 3<sup>rd</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>5</sup>Specific PCR fragments shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR bands.

<sup>6</sup>Individual alleles can give to rise to two differently sized specific PCR fragments in primer mixes 49, 51, 53 to 56, 58, 59, 61, 62, 66 to 68, 70, 75 and 77.

<sup>7</sup>Primer mixes 49, 67 and 70 have a tendency to giving rise to primer oligomer formation.

<sup>8</sup>Primer mixes 51 and 52 may have tendencies of unspecific amplifications.

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<sup>9</sup>Primer mixes 67 and 72 has a tendency of primer oligomer formation and also has an intense primer cloud due to the high number of primers present in the primer mix.

<sup>10</sup>Due to sharing of sequence motifs in codon 38, DRB3\*01:14 will also be amplified in primer mixes 53, 54 and 65 in addition to primer mix 85.

<sup>11</sup>The DRB4\*01:03:01:02N allele is amplified by primer mixes 66 and 78, whereas the DRB4\*02:01N and DRB4\*03:01N null alleles are not amplified by this primer pair.

<sup>12</sup>Primer mix 54 may give a lower yield of HLA-specific PCR products than the other DRB primer mixes.

<sup>13</sup>Primer mix 96 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.

‘w’, might be weakly amplified.

‘?’, nucleotide sequence information not available for the primer matching sequence.

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## DQA1 PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	145	170	145	170	220	100	95	65	175	185	215	225
Length of int. pos. control <sup>1</sup>	515	515	430	430	430	430	430	430	430	430	430	515
5'-primer(s) <sup>2</sup>	33(169)	25(143)	33(169)	25(143)	-7(49)	198(664)	25(143)	-7(49)	7(90)	7(90)	-6(53)	99(366)
	5' -Agg 3'	5' -gTA 3'	5' -AgC 3'	5' -gTT 3'	5' -CCA 3'	5' -gCA 3'	5' -gTA 3'	5' -CCg 3'	5' -CAC 3'	5' -CAT 3'	5' -gAC 3'	5' -CCC 3'
3'-primer(s) <sup>3</sup>	68(274)	68(274)	68(274)	68(274)	1 <sup>st</sup> I	218(722)	43(199)	2(74)	52(224)	54(232)	1 <sup>st</sup> I	160(548)
	5' -TgC 3'	5' -TgC 3'	5' -TgC 3'	5' -TgC 3'	5' -TTT 3'	5' -CTT 3'	5' -AgC 3'	5' -TgT 3'	5' -TgT 3'	5' -TCT 3'	5' -TTT 3'	5' -CAT 3'

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	13	14	15	16	17	18	19	20	21	22	23	24
Length of int. pos. control <sup>1</sup>	225	125	165	95	200	200	205	135	100	120	85	220
5'-primer(s) <sup>2</sup>	515	430	430	515	430	430	430	430	430	515	430	430
	99(366)	25(143)	33(169)	59(245)	107(389)	107(389)	21(131)	21(131)	-13(31)	25(143)	32(165)	up <sup>4</sup>
	5' -CCC 3'	5' -gTA 3'	5' -AgC 3'	5' -CCg 3'	5' -CAT 3'	5' -CAT 3'	5' -TCC 3'	5' -TCT 3'	5' -ggA 3'	5' -gTT 3'	5' -gAC 3'	5' -ACT 3'
				188(634)					103(377)			
				5' -CTA 3'					5' -ggA 3'			
3'-primer(s) <sup>3</sup>	160(548)	52(226)	75(293)	75(293)	159(547)	159(547)	75(293)	51(223)	1 <sup>st</sup> I	51(223)	46(208)	-13(31)
	5' -CAG 3'	5' -TTg 3'	5' -gAC 3'	5' -gAC 3'	5' -AgC 3'	5' -AgA 3'	5' -gAC 3'	5' -TCT 3'	5' -TgC 3'	5' -TCT 3'	5' -ACA 3'	5' -ggC 3'
	160(548)			207(691)					160(548)	83(319)		
	5' -CAG 3'			5' -gCA 3'					5' -CAG 3'	5' -AgT 3'		

Well No.	25	26	27	28	29	30	31
Length of spec. PCR product	80	160	90	105	150	115	135
Length of int. pos. control <sup>1</sup>	175		135		250	215	
5'-primer(s) <sup>2</sup>	430	430	430	430	430	430	430
	-13(31)	33(169)	52(226)	152(526)	99(366)	101(373)	107(389)
	5' -ggA 3'	5' -AgC 3'	5' -gAT 3'	5' -gTC 3'	5' -CCC 3'	5' -CAG 3'	5' -CAT 3'
	33(169)	101(372)	186(626)			194(650)	
	5' -Agg 3'	5' -ACg 3'	5' -TCT 3'			5' -Agg 3'	
3'-primer(s) <sup>3</sup>	0(70)	76(298)	68(274)	174(591)	134(470)	159(547)	139(485)
	5' -TTT 3'	5' -AAC 3'	5' -TgT 3'	5' -TCg 3'	5' -CAG 3'	5' -AgA 3'	5' -AgA 3'
	78(304)	138(482)	218(722)		138(484)	218(722)	
	5' -gCA 3'	5' -TgA 3'	5' -CTT 3'		5' -gCg 3'	5' -CTC 3'	
				168(574)			
				5' -CTg 3'			

<sup>1</sup>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.

<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

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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.

<sup>4</sup>Primer located upstream of the 1<sup>st</sup> exon.

## DQB1 PRIMER SPECIFICATION

Well No.	32	33	34	35	36	37	38	39	40	41	42	43
Length of spec. PCR product	135 230  220  270	140 185  220  205	210  220	220	130  220	135	85	160  210	170	215  185	185	185
Length of int. pos. control <sup>1</sup>	515  515	515  430	515  515	515  515	515  515	430  430	430  515	430  430	430  430	430  430	430  430	430
5'-primer(s) <sup>2</sup>	25(170) 5' -gCA 3'  26(173) 5' -ggg 3'  26(173) 5' -TgT 3'  26(173) 5' -TTA 3'  26(173) 5' -TCT 3'	9(122) 5' -gTT 3'  24(169) 5' -Ag 3'  30(185) 5' -AAg 3'  28(179) 5' -gAC 3'  26(173) 5' -ACC 3'  71(309) 5' -gCT 3'  23(164) 5' -ggg 3'  26(173) 5' -gAC 3'  29(184) 5' -ACC 3'  21(159) 5' -gCA 3'	29(184) 5' -gAg 3'  26(173) 5' -TTA 3'  28(179) 5' -gAC 3'  26(173) 5' -ACC 3'  71(309) 5' -gCT 3'  26(173) 5' -gCg 3'  29(184) 5' -gAC 3'  21(159) 5' -gCA 3'	26(173) 5' -ggg 3'  26(173) 5' -TgT 3'  26(173) 5' -TTA 3'  26(173) 5' -TCT 3'	86(353) 5' -gCT 3'  86(354) 5' -AgT 3'  86(353) 5' -ggT 3'  86(356) 5' -ggT 3'  86(361) 5' -CCT 3'  86(354) 5' -AAA 3'  86(354) 5' -AAg 3'	86(353) 5' -Cgg 3'  57(266) 5' -Cgg 3'  86(354) 5' -AgT 3'  57(266) 5' -Cgg 3'  86(356) 5' -ggg 3'  86(354) 5' -AgT 3'  86(355) 5' -gAC 3'	71(309) 5' -TCT 3'  38(210) 5' -gCg 3'  71(309) 5' -gCT 3'  38(210) 5' -gCg 3'  26(173) 5' -gAC 3'  29(184) 5' -ACC 3'  21(159) 5' -gCA 3'	26(173) 5' -gAT 3'  29(184) 5' -gAC 3'  29(184) 5' -gAC 3'  29(184) 5' -gAT 3'	77(327) 5' -gCT 3'  69(304) 5' -ACg 3'  87(356) 5' -CTC 3'  69(304) 5' -CCT 3'  86(353) 5' -gCT 3'	69(304) 5' -ACg 3'  87(356) 5' -ggT 3'  69(304) 5' -CCT 3'  86(353) 5' -gCT 3'	87(356) 5' -ggT 3'  87(358) 5' -gCC 3'  86(354) 5' -gCC 3'	86(353) 5' -gCT 3'
Well No.	32	33	34	35	36	37	38	39	40	41	42	43

Well No.	44	45	46	47	48
Length of spec. PCR product	220  210	190  210	160  210	210	210
Length of int. pos. control <sup>1</sup>	515  430	515  515	515  515	515  430	430
5'-primer(s) <sup>2</sup>	26(173) 5' -TTA 3'  86(353) 5' -ACg 3'	9(122) 5' -gTT 3'  57(266) 5' -CAT 3'	29(184) 5' -gAC 3'  69(304) 5' -CCC 3'	29(184) 5' -gAC 3'  86(353) 5' -ACC 3'	29(184) 5' -gAC 3'  86(353) 5' -ACC 3'
3'-primer(s) <sup>3</sup>	26(173) 5' -gAT 3'  58(270) 5' -TCC 3'	29(184) 5' -gAC 3'  86(353) 5' -ACg 3'	29(184) 5' -gAC 3'  86(354) 5' -TAT 3'	29(184) 5' -gAC 3'  86(354) 5' -TAT 3'	29(184) 5' -gAC 3'
Well No.	44	45	46	47	48

<sup>1</sup>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 32 contains the longer, 515 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the

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**Lot No.: 17X**

#### Lot-specific information

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.

<sup>4</sup>Primer located upstream of the 1<sup>st</sup> exon.

## DRB PRIMER SPECIFICATION

Well No.	49	50	51	52	53	54	55	56	57	58	59	60	
Length of spec.	210	200	210	210	120	75	85	100	235	170	90	180	
PCR product	235		230		220	210	210	175		215	135		
			260							250	190		
Length of int. pos. control <sup>1</sup>	515	430	430	430	430	430	430	430	430	515	430	430	
5'-primer(s) <sup>2</sup>	12(124)	14(129)	13(126)	13(126)	13(125)	13(125)	13(125)	13(125)	13(127)	15(133)	26(165)	26(164)	
	5' -A.T 3'	5' -gAA 3'	5' -AgG 3'	5' -AgG 3'	5' -gTC 3'	5' -gTC 3'	5' -gTC 3'	5' -ACA 3'	5' -ATA 3'	5' -gTT 3'	5' -TAT 3'	5' -gTA 3'	
	14(129)		13(126)	13(126)	47(227)	15(133)			13(125)	13(127)	15(133)	58(261)	30(178)
	5' -gAA 3'		5' -AAg 3'	5' -AAg 3'	5' -gTT 3'	5' -gTT 3'			5' -ACC 3'	5' -ATA 3'	5' -gTT 3'	5' -gAg 3'	5' -gCg 3'
			13(126)						13(125)	13(127)	15(133)		
			5' -AgA 3'						5' -ATA 3'	5' -gTA 3'	5' -gCT 3'		
									13(125)				
									5' -gTC 3'				
3'-primer(s) <sup>3</sup>	66(286)	66(286)	66(286)	66(286)	73(305)	26(164)	28(171)	32(184)	77(319)	58(260)	57(257)	73(307)	
	5' -gAg 3'	5' -gAT 3'	5' -gAT 3'	5' -gAA 3'	5' -ggC 3'	5' -ggT 3'	5' -CTC 3'	5' -gTg 3'	5' -CAC 3'	5' -CCT 3'	5' -CgA 3'	5' -CgC 3'	
	66(286)		69(295)	66(286)	73(305)	71(299)	69(295)	58(260)	77(319)	73(307)	73(305)		
	5' -gAg 3'		5' -CTg 3'	5' -gAg 3'	5' -ggC 3'	5' -gCT 3'	5' -CTg 3'	5' -Cgg 3'	5' -gTA 3'	5' -CAg 3'	5' -ggC 3'		
	66(286)		69(295)	70(297)	73(305)				77(319)	86(344)	77(319)		
	5' -gAT 3'		5' -Tg 3'	5' -CTg 3'	5' -ggC 3'				5' -CAA 3'	5' -CAC 3'	5' -CAC 3'		
	70(297)		70(298)	71(301)	74(308)								
	5' -CTg 3'		5' -CgC 3'	5' -ggC 3'	5' -CCC 3'								
	71(299)		71(299)		74(310)								
	5' -gCg 3'		5' -gCT 3'		5' -CAA 3'								
	77(317)		73(305)										
	5' -AgT 3'		5' -ggC 3'										
	86(344)		77(317)										
	5' -CCA 3'		5' -AgT 3'										
Well No.	49	50	51	52	53	54	55	56	57	58	59	60	

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 101.713-24u/06u – without *Taq* polymerase, IFU-02

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## Lot-specific information

Well No.	61	62	63	64	65	66	67	68	69	70	71	72
Length of spec.	100	90	220	200	175	100	110	110	165	75	135	180
PCR product	170	110		225		150	145	150		175		
						195	170	180		265		
						240		220				
Length of int. pos. control <sup>1</sup>	430	430	430	430	430	430	430	430	430	430	430	430
5'-primer(s) <sup>2</sup>	13(125) 5' -gTC 3' 15(133) 5' -gTC 3' 38(200) 5' -CgT 3'	12(124) 5' -Cgg 3' 15(133) 5' -gTT 3' 13(125) 5' -gTC 3'	10(116) 5' -gCT 3' 12(122) 5' -TAT 3' 13(125) 5' -gTC 3'	10(116) 5' -gCT 3' 12(122) 5' -TAT 3' 13(125) 5' -gTC 3'	13(125) 5' -gTC 3' 37(197) 5' -gTT 3' 38(200) 5' -CgT 3'	1 <sup>st</sup> I 5' -CAA 3' 37(197) 5' -gTA 3'	26(164) 5' -gTA 3' 37(197) 5' -gTA 3'	13(125) 5' -gTC 3' 34(189) 5' -CAg 3' 37(197) 5' -gTA 3'	15(133) 5' -gTT 3' 34(189) 5' -CAg 3' 36(196) 5' -AgC 3'	12(122) 5' -TAG 3' 15(133) 5' -gCT 3' 15(133) 5' -gTC 3'	15(133) 5' -gTT 3' 74(308) 5' -CCT 3' 107(409) 5' -AgA 3'	13(125) 5' -gTC 3'
3'-primer(s) <sup>3</sup>	58(260) 5' -CCT 3' 58(260) 5' -CCT 3' 58(260) 5' -CCT 3'	29(175) 5' -gTg 3' 37(199) 5' -CAg 3' 71(299) 5' -gCT 3'	69(295) 5' -gTC 3' 70(298) 5' -CgC 3' 70(298) 5' -ACT 3'	66(286) 5' -gAA 3' 58(260) 5' -Cgg 3' 70(298) 5' -CgC 3'	58(260) 5' -Cgg 3' 58(260) 5' -Cgg 3' 70(298) 5' -Cgg 3'	42(213) 5' -TCA 3' 57(257) 5' -CAg 3' 70(298) 5' -Cgg 3'	57(257) 5' -CAg 3' 57(257) 5' -CAg 3' 70(298) 5' -Cgg 3'	57(257) 5' -CAg 3' 59(265) 5' -gTg 3' 70(298) 5' -Cgg 3'	56(256) 5' -gCT 3' 57(257) 5' -gTg 3' 70(298) 5' -Cgg 3'	56(256) 5' -gCT 3' 86(344) 5' -CAT 3' 70(298) 5' -Cgg 3'	47(227) 5' -ggA 3' 159(565) 5' -CAT 3'	56(256) 5' -gCT 3'
Well No.	61	62	63	64	65	66	67	68	69	70	71	72

Well No.	73	74	75	76	77	78	79	80	81	82	83	84
Length of spec.	150	145	145	140	160	215	175	215	215	225	170	75
PCR product				210		240						
				235								
Length of int. pos. control <sup>1</sup>	430	430	430	430	430	430	430	515	430	430	430	430
5'-primer(s) <sup>2</sup>	34(189) 5' -CAg 3' 34(189) 5' -CAg 3'	13(125) 5' -gTC 3' 37(197) 5' -gTT 3'	13(125) 5' -gTC 3' 10(116) 5' -gAT 3' 114(429) 5' -CTg 3'	36(196) 5' -AgA 3' 10(116) 5' -gCT 3' 37(199) 5' -TCC 3'	10(116) 5' -gCT 3' 28(170) 5' -gAT 3' 37(199) 5' -TCC 3'	28(170) 5' -gAT 3' 13(125) 5' -gTA 3' 37(199) 5' -TCC 3'	13(125) 5' -gTA 3' 13(125) 5' -ACA 3' 37(199) 5' -TCC 3'	13(125) 5' -ACA 3' 13(125) 5' -ACA 3' 37(199) 5' -TCC 3'	13(125) 5' -ACA 3' 13(125) 5' -ACA 3' 37(199) 5' -TCC 3'	13(125) 5' -ACA 3' 13(125) 5' -ACA 3' 74(308) 5' -CgA 3'	74(308) 5' -CgA 3'	74(308) 5' -CgA 3'
3'-primer(s) <sup>3</sup>	70(298) 5' -CTC 3' 47(229) 5' -CCA 3'	47(227) 5' -ggA 3' 73(307) 5' -CAg 3'	70(296) 5' -TCC 3' 70(298) 5' -CTg 3'	69(295) 5' -CTg 3' 77(317) 5' -CCC 3'	51(239) 5' -CTg 3' 86(346) 5' -CTC 3'	86(346) 5' -CTC 3' 57(258) 5' -gCg 3'	57(258) 5' -gCg 3' 70(298) 5' -CTT 3'	70(298) 5' -CTT 3' 70(298) 5' -CTC 3'	70(298) 5' -CTC 3' 74(308) 5' -CCT 3'	74(308) 5' -CCT 3' 56(256) 5' -gCT 3'	56(256) 5' -gCT 3'	
Well No.	73	74	75	76	77	78	79	80	81	82	83	84

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Lot No.: 17X

## Lot-specific information

Well No.	85	86	87	88	89	90	91	92	93	94	95
Length of spec.	250	210	135	165	95	120	155	130	100	185	195
PCR product											
Length of int.	515	430	430	430	430	430	430	430	430	430	430
pos. control <sup>1</sup>											
5'-primer(s) <sup>2</sup>	111(421) 5' -ACT 3' 13(125) 5' -gTC 3' 47(227) 5' -gTT 3' 10(116) 5' -gCT 3' 11(119) 5' -gCg 3' 11(119) 5' -gCT 3' 10(116) 5' -gCT 3' 11(119) 5' -gTg 3'										
3'-primer(s) <sup>3</sup>	181(630) 5' -CTT 3' 70(296) 5' -TCC 3' 78(321) 5' -CAA 3' 51(239) 5' -CCC 3' 28(171) 5' -CTg 3' 37(197) 5' -Cgg 3' 42(213) 5' -TCA 3' 134(490) 5' -gCT 3' 57(258) 5' -gCg 3' 85(341) 5' -CAg 3' 159(565) 5' -CAT 3'										
Well No.	85	86	87	88	89	90	91	92	93	94	95

<sup>1</sup>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 49 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.

<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.





101.713-24/06 – including *Taq* polymerase, IFU-01  
101.713-24u/06u – without *Taq* polymerase, IFU-02

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**Lot No.: 17X****Lot-specific information**

<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 7, 16, 20 and 26 to 29 were available. The specificities of the primers in primer solution 16 were tested by separately adding one 5'-primer, respectively one 3'-primer.

In primer solutions 7, 26 and 29 the 5'-primers were tested by adding one additional 3'-primer, the 3'-primers were not possible to test. In primer solutions 20, 27 and 28 the 3'-primers were tested by adding one additional 5'-primer, the 5'-primers were not possible to test. In primer solutions 16, 21 and 30, one 5'-primer was not possible to test, and in primer solution 16 22 and 25 one 3'-primer was not possible to test. Additional 3'-primers in primer solutions 21 and 30 were tested by separately adding one 5'-primer, and one additional 5'-primer in primer solution 25 was tested by separately adding one 3'-primer.



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101.713-24u/06u – without *Taq* polymerase, IFU-02

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Lot No.: 17X

**Lot-specific information**

<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.

In primer solutions 32, 33 and 34 one 5'-primer was not possible to test, and in primer solutions 32, 33, 35, 36, 38, 45 and 47 one or two 3'-primers were not possible to test.

Additional 5'-primers in primer solutions 33 and 38 were tested by separately adding another 3'-primer.

Additional 3'-primers in primer solutions 32 and 33 were tested by separately adding one 5'-primer.



101.713-24/06 – including *Taq* polymerase, IFU-01  
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Lot No.: 17X

Lot-specific information

<b>CELL LINE VALIDATION SHEET</b>															
DRB resolution primer set <sup>3</sup>															
Prod. No.:	Well <sup>2</sup>														
	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79
<b>IHWC cell line<sup>1</sup></b>	<b>DRB1</b>														
1 9001 SA	*01:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2 9280 LK707	*15:02	*04:05	-	-	-	-	-	-	-	-	-	-	+ +	-	
3 9011 E4181324	*15:02	-	-	-	-	-	-	-	-	-	-	-	-	+ +	
4 9275 GU373	*03:01	+ -	-	-	-	-	-	-	-	-	-	-	-	-	
5 9009 KAS011	*16:01	-	-	-	-	-	-	-	-	-	-	-	-	+ -	
6 9353 SM	*04:07	*08:03	-	-	-	+	-	-	-	-	-	-	-	+ -	
7 9020 QBL	*03:01	+ -	-	-	-	-	-	-	-	-	-	-	-	-	
8 9025 DEU	*04:01	-	-	-	-	-	-	-	-	-	-	-	-	+ - +	
9 9026 YAR	*04:02	-	-	-	-	-	-	-	-	-	-	-	-	-	
10 9107 LKT3	*04:05	-	-	-	-	-	-	-	-	-	-	-	-	-	
11 9051 PITOUT	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	
12 9052 DBB	*07:01	-	+	-	-	-	-	-	-	-	-	-	-	+ -	
13 9004 JESTHOM	*01:01	-	-	-	-	-	-	-	-	-	-	-	-	-	
14 9071 OLGA	*08:02	-	-	-	+	-	-	-	-	-	-	-	-	-	
15 9075 DKB	*09:01	-	-	+	-	-	-	-	-	-	-	-	-	+ -	
16 9037 SWEIG007	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	+ -	
17 9282 CTM3953540	*03:01	*13:01	+ -	-	-	-	-	-	-	-	-	-	-	+ -	
18 9257 32367	*09:01	*11:01	-	-	+	-	-	-	-	-	-	-	-	+ +	
19 9038 BM16	*12:01	-	-	-	-	-	-	+	-	-	-	-	-	+ -	
20 9059 SLE005	*13:02	+ -	-	-	-	-	-	-	-	-	-	-	-	+ -	
21 9064 AMALA	*14:02	+ -	+	-	-	-	-	-	-	-	-	-	-	+ + -	
22 9056 KOSE	*13:02	*14:54	+ +	+	+	-	-	-	+ +	+ +	-	+ +	-	-	
23 9124 IHL	*08:03	*14:14	+ -	+	+	+	-	-	-	-	-	+ -	-	-	
24 9035 JBUSH	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	
25 9049 IBW9	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	+ -	
26 9285 WT49	*03:01	+ -	-	-	-	-	-	-	-	-	-	-	-	+ -	
27 9191 CH1007	*04:05	*10:01	-	-	-	-	-	-	-	-	-	-	-	+ -	
28 9320 BEL5GB	*04:16	*07:01	-	-	-	-	-	-	-	-	-	-	-	+ - +	
29 9050 MOU	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	+ -	
30 9021 RSH	*03:02	+ -	-	-	-	-	-	-	-	-	-	-	-	+ -	
31 9019 DUCAF	*03:01	+ -	-	-	-	-	-	-	-	-	-	-	-	+ -	
32 9297 HAG	*13:03	+ -	-	-	-	-	-	-	-	-	-	-	-	+ -	
33 9098 MT14B	*04:04	-	-	-	-	-	-	-	-	-	-	-	-	+ -	
34 9104 DHIF	*11:01	-	-	-	-	-	-	-	-	-	-	-	-	+ -	
35 9302 SSTO	*04:03	-	-	-	-	-	-	-	-	-	-	-	-	+ -	
36 9024 KT17	*04:03	*04:06	-	-	-	-	-	-	-	-	-	-	-	+ -	
37 9065 HHKB	*13:01	+ -	-	-	-	-	-	-	-	-	-	-	-	-	
38 9099 LZL	*14:02	+ -	+	-	-	-	-	-	-	-	-	-	-	+ -	
39 9315 CML	*03:01	*04:01	+ -	-	-	-	-	-	-	-	-	-	-	+ -	
40 9134 WHONP199	*07:01	*09:01	-	-	+	-	-	-	-	-	-	-	-	+ -	
41 9055 H0301	*13:02	+ -	-	-	-	-	-	-	-	-	-	-	-	+ -	
42 9066 TAB089	*08:03	-	-	-	-	+	-	-	-	-	-	-	-	-	
43 9076 T7526	*09:01	-	-	+	-	-	-	-	-	-	-	-	-	+ -	
44 9057 TEM	*14:01	-	+	+	+	-	-	-	-	-	-	-	-	-	
45 9239 SHJO	*07:01	-	-	-	-	-	-	-	-	-	-	-	-	+ -	
46 9013 SCHU	*15:01	-	-	-	-	-	-	-	-	-	-	-	-	+ -	
47 9045 TUBO	*11:04	*12:01	-	-	-	-	-	+	-	-	-	-	-	+ -	
48 9303 TER-ND	*01:03	-	-	-	-	-	-	-	-	-	-	-	-	-	



101.713-24/06 – including *Taq* polymerase, IFU-01  
101.713-24u/06u – without *Taq* polymerase, IFU-02

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Lot No.: 17X

#### Lot-specific information

<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 DRB4\*01:03:01:02N allele is amplified by primer mix 66 in the DBB/9052 cell line.

<sup>3</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.

Additional 5'- and 3'-primers in primer solutions 59, 66 and 75 were tested by separately adding one 3'-primer, respectively one 5'-primer.

Additional 5'-primers in primer solutions 54, 60, 63 and 64 were tested by separately adding one 3'-primer. Additional 3'-primers in primer solutions 49, 51, 52, 57, 58, 68, 70 and 89 were tested by separately adding one 5'-primer. One, two or three of the 5'-primers in primer solution 49, 51, 52, 56 to 58, 61 to 64, 68, 70, 75 and 89 were not possible to test. One or two of the 3'-primers in primer solution 49, 51, 52, 53, 57, 61, 63, 69, 74, 78 and 89 were not possible to test.

101.713-24/06 – including *Taq* polymerase, IFU-01  
101.713-24u/06u – without *Taq* polymerase, IFU-02

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**Lot No.: 17X**

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