

101.215-12 – including *Taq* pol., IFU-01  
101.215-12u – without *Taq* pol., IFU-02

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“Instructions for Use” (IFU)

Lot No.: **26Y**

Lot-specific Information  
**Olerup SSP® DQB1\*04**

Product number:	101.215-12 – including <i>Taq</i> polymerase 101.215-12u – without <i>Taq</i> polymerase
Lot number:	26Y
Expiry date:	2017-November-01
Number of tests:	12
Number of wells per test:	15+1
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

**This Product Description is only valid for Lot No. 26Y.**

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

**CHANGES COMPARED TO THE PREVIOUS OLERUP SSP®  
DQB1\*04 Lot (47V)**

The DQB1\*04 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.

One well has been added to DQB1\*04, well **16**.

The DQB1\*04 primer set, specificity and interpretation tables have been updated with the DQB1 alleles described since the previous *Olerup SSP®* DQB1\*04 lot (**Lot No. 47V**) was made. The kit design is based on IMGT/HLA database 3.19.0.

As of lot series V, the Specificity Table is included in the lot-specific Product Insert, and the Interpretation Table is included in the Worksheet.

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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
1	Exchanged	Exchanged	5'-primers and 3'-primer exchanged for improved yield.
3	-	Exchanged	3'-primer exchanged for the DQB1*04:02:08 allele.
6	Added	-	5'-primer added for the DQB1*04:20 allele.
7	Moved, added	Moved, added	Primer pair moved to well 12, primer pair added for the DQB1*04:02:08 allele.
11	Added	Added	Primer pair added for the DQB1*04:23 allele.
12	Added	Added	Primer pair added from well 7.
15	Added	Added	Updated negative control moved to well 16, primer pairs added for the DQB1*04:23 and 04:25N alleles.
16	-	-	Updated negative control added from well 15.

Changes in revision R01 compared to R00:

1. The Product Insert and Worksheet have been corrected to reflect the resolution capability of the kit. As of lot series “Y”, the DQB1\*04 kit enables separation of the confirmed DQB1\*04 alleles as listed in the IMGT/HLA database, polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.

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

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

Length of PCR product	105	200	105	80	75	80	85
<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>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 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

### DQB1\*04 SSP subtyping

#### CONTENT

The primer set contains 5'- and 3'-primers for identifying the DQB1\*04:01 to DQB1\*04:27 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 16 PCR reactions in a 16 well PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	NC

The 16 well PCR plate is marked with 'DQB1\*04' in silver/gray ink.

Well No. 1 is marked with the Lot No. '26Y'.

Wells 1 to 15 – DQB1\*04 high resolution primers.

Well 16 – Negative Control (NC).

A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.

The PCR plates are covered with a PCR-compatible foil.

**Please note:** When removing each 16 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 DQB1 alleles non-DQB1\*04 alleles will be amplified by primer mixes 1, 4, 5, 7, 8, 10, 11 and 15.

Thus, the interpretation of DQB1\*04 subtypings is not influenced by other groups of the DQB1 alleles or the DQB2 and DQB3 genes.

For further details see Specificity Table.

#### UNIQUELY IDENTIFIED ALLELES

All the DQB1\*04 alleles, i.e. **DQB1\*04:01 to DQB1\*04:27**, recognized by the HLA Nomenclature Committee in January 2015<sup>1,2</sup> will be amplified by the primers in the DQB1\*04 subtyping kit.

The DQB1\*04 kit enables separation of the confirmed DQB1\*04 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 DQB1\*04 alleles is listed below.

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The DQB1\*04 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 DQB1\*04 alleles can be distinguished by the different sizes of the specific PCR product:

Alleles	Primer mix
DQB1*04:04, 04:12	5
DQB1*04:05, 04:06	5

The DQB1\*04 subtyping kit cannot distinguish the silent mutations in the DQB1\*04:01:01-04:01:04 alleles or the DQB1\*04:02:01-04:02:07 alleles.

<sup>1</sup>HLA-DQB1 alleles listed on the IMGT/HLA web page 2015-January-19, release 3.19.0, [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>.

**RESOLUTION IN HOMO- AND HETEROZYGOTES**

Results file with resolution in DQB1\*04 homo- and heterozygotes is available upon request.

**ALLELE CONFIRMATION STATUS**

Allele	Status <sup>1</sup>	Allele	Status <sup>1</sup>
<b>DQB1*04:01:01</b>	<b>Confirmed</b>	DQB1*04:10	Unconfirmed
DQB1*04:01:02	Unconfirmed	<b>DQB1*04:11</b>	<b>Confirmed</b>
DQB1*04:01:03	Unconfirmed	<b>DQB1*04:12</b>	<b>Confirmed</b>
DQB1*04:01:04	Unconfirmed	DQB1*04:13	Unconfirmed
<b>DQB1*04:02:01</b>	<b>Confirmed</b>	DQB1*04:14	Unconfirmed
DQB1*04:02:02	Unconfirmed	DQB1*04:15	Unconfirmed
<b>DQB1*04:02:03</b>	<b>Confirmed</b>	DQB1*04:16	Unconfirmed
<b>DQB1*04:02:04</b>	<b>Confirmed</b>	DQB1*04:17	Unconfirmed
DQB1*04:02:05	Unconfirmed	<b>DQB1*04:18</b>	<b>Confirmed</b>
DQB1*04:02:06	Unconfirmed	DQB1*04:19	Unconfirmed
<b>DQB1*04:02:07</b>	<b>Confirmed</b>	DQB1*04:20	Unconfirmed
DQB1*04:02:08	Unconfirmed	DQB1*04:21	Unconfirmed
DQB1*04:03:01	Unconfirmed	DQB1*04:22	Unconfirmed
DQB1*04:03:02	Unconfirmed	DQB1*04:23	Unconfirmed
DQB1*04:04	Unconfirmed	DQB1*04:24	Unconfirmed
DQB1*04:05	Unconfirmed	DQB1*04:25N	Unconfirmed
DQB1*04:06	Unconfirmed	DQB1*04:26	Unconfirmed
DQB1*04:07	Unconfirmed	DQB1*04:27	Unconfirmed
DQB1*04:08	Unconfirmed		
DQB1*04:09	Unconfirmed		

<sup>1</sup>Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2015-January-19, release 3.19.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

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**SPECIFICITY TABLE**

**DQB1\*04 SSP subtyping**

Specificities and sizes of the PCR products of the 15+1 primer mixes used for DQB1\*04 SSP subtyping

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified DQB1*04 alleles <sup>3</sup>	Amplified non-DQB1*04 alleles <sup>4</sup>
<b>1<sup>6</sup></b>	160 bp	<b>515 bp</b>	*04:01:01-04:01:02, 04:01:04-04:02:07, 04:03:01-04:27	*03:132
	205 bp		*04:01:01-04:01:04, 04:05- 04:08, 04:14-04:17	
<b>2<sup>6</sup></b>	205 bp	<b>515 bp</b>	*04:01:01-04:01:04, 04:05- 04:08, 04:14-04:17	
<b>3</b>	180 bp	430 bp	*04:02:01-04:03:01, 04:04, 04:09-04:13, 04:18-04:19, 04:21, 04:22 <sup>w</sup> , 04:23, 04:24 <sup>w</sup> , 04:25N-04:27	
<b>4</b>	195 bp	430 bp	*04:03:01-04:03:02	*03:06, 03:25
<b>5<sup>5</sup></b>	110 bp	430 bp	*04:06, 04:12	
	245 bp		*04:04-04:05	*03:06, 03:25
<b>6<sup>5</sup></b>	95 bp	430 bp	*04:16	
	210 bp		*04:20	
<b>7<sup>8</sup></b>	245 bp	430 bp	*04:02:08	*03:30, 03:72, 03:100, 06:02:01-06:02:23, 06:05:02, 06:10, 06:13:01-06:16, 06:19:01-06:20, 06:22:01- 06:24, 06:29, 06:33, 06:37, 06:46-06:51:02, 06:68-06:84, 06:95-06:97, 06:106-06:107, 06:109, 06:111-06:117, 06:119, 06:122-06:123, 06:125-06:127, 06:130, 06:136, 06:138-06:139, 06:146-06:147, 06:150-06:152, 06:156, 06:159, 06:161- 06:163, 06:166
<b>8<sup>5</sup></b>	95 bp	430 bp	*04:08	*03:06 <sup>?</sup> -03:08 <sup>?</sup> , 03:10:02 <sup>?</sup> - 03:15 <sup>?</sup> , 03:17:01 <sup>?</sup> -03:18 <sup>?</sup> , 03:19, 03:20 <sup>?</sup> , 03:23 <sup>?</sup> , 03:26 <sup>?</sup> , 03:37 <sup>?</sup> , 03:40 <sup>?</sup> , 03:48 <sup>?</sup> , 03:52 <sup>?</sup> - 03:71 <sup>?</sup> , 03:74 <sup>?</sup> -03:78 <sup>?</sup> , 03:81 <sup>?</sup> - 03:82 <sup>?</sup> , 03:101 <sup>?</sup> -03:112 <sup>?</sup> , 03:118N <sup>?</sup> -03:163 <sup>?</sup> , 03:165 <sup>?</sup> - 03:167 <sup>?</sup>
<b>9</b>	140 bp	430 bp	*04:09, 04:14	
<b>10</b>	145 bp	430 bp	*04:10	*05:01:19 <sup>w</sup> , 06:03:03 <sup>w</sup>
<b>11<sup>5</sup></b>	120 bp	430 bp	*04:11, 04:15	
	160 bp		*04:23	*03:22, 03:96

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<b>12</b>	160 bp 230 bp	430 bp	*04:07 *04:18	
<b>13</b>	185 bp	430 bp	*04:13	
<b>14<sup>7</sup></b>	160 bp	430 bp	*04:17	
<b>15</b>	150 bp	430 bp	*04:23, 04:25N	*03:22, 03:96
<b>16<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 DQB1\*04 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 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>Due to the sharing of sequence motifs between DQB1 alleles non-DQB1\*04 alleles will be amplified by primer mixes 1, 4, 5, 7, 8, 10, 11 and 15.

<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 mix 2 may give rise to a lower yield of HLA-specific PCR product than the other DQB1\*04 primer mixes.

<sup>7</sup>Primer mix 14 may have tendencies of unspecific amplifications.

<sup>8</sup>In primer mix 7 the positive control band may be weaker than for other DQB1\*04 primer mixes.

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

‘w’, might be weakly amplified.

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

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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	160	205	180	195	110	95	245	95	140	145	120	160
	205				245	210					160	230
Length of int. pos. control <sup>1</sup>	515	515	430	430	430	430	430	430	430	430	430	430
5'-primer(s) <sup>2</sup>	23(164) 5'-gCT 3'	23(164) 5'-gCT 3'	23(164) 5'-gCg 3'	26(173) 5'-TCT 3'	9(122) 5'-gTA 3'	21(160) 5'-CCA 3'	9(122) 5'-gTT 3'	167(596) 5'-gCA 3'	43(226) 5'-ACA 3'	23(164) 5'-gCg 3'	48(241) 5'-ggA 3'	15(140) 5'-gTA 3'
	38(210) 5'-gCg 3'		23(164) 5'-gCg 3'		54(259) 5'-ggT 3'	59(272) 5'-CgT 3'			45(230) 5'-ggA 3'		52(251) 5'-gCT 3'	144(529) 5'-CCg 3'
											130(485) 5'-CCA 3'	
3'-primer(s) <sup>3</sup>	77(327) 5'-ACg 3'	77(327) 5'-ACg 3'	69(304) 5'-CTC 3'	77(327) 5'-ACg 3'	77(327) 5'-ACg 3'	77(327) 5'-ACg 3'	77(326) 5'-CCg 3'	185(650) 5'-CgA 3'	77(327) 5'-ACg 3'	57(267) 5'-gCA 3'	77(327) 5'-ACg 3'	77(327) 5'-ACg 3'
											169(604) 5'-gAC 3'	185(650) 5'-CgA 3'
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15
Length of spec. PCR product	185	160	150
Length of int. pos. control <sup>1</sup>	430	430	430
5'-primer(s) <sup>2</sup>	139(514) 5'-CAA 3'	146(533) 5'-CCT 3'	26(173) 5'-ggg 3'
			130(485) 5'-CCA 3'
3'-primer(s) <sup>3</sup>	187(656) 5'-ACA 3'	185(650) 5'-CgA 3'	58(271) 5'-CTA 3'
			169(604) 5'-gAC 3'
Well No.	13	14	15

<sup>1</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 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 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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<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.

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<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 4 to 6 and 9 to 15 were available. The specificities of the primers in primer solutions 4, 5, 9 and 15 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solutions 6, 11, 12 and 14 it was only possible to test the 3'-primer, the 5'-primers were not possible to test. In primer solutions 10 and 13 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. In primer solutions 3, 5, 9 and 15 one 5'-primer was not possible to test.

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Lot-specific Information

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