

101.211-24 – including *Taq* polymerase
101.211-24u – without *Taq* polymerase

Visit www.caredx.com for
“Instructions for Use” (IFU)

Lot No.: **7K2**

Lot-specific information
Olerup SSP® DQB1*05

Product number:	101.211-24 – including <i>Taq</i> polymerase 101.211-24u – without <i>Taq</i> polymerase
Lot number:	7K2
Expiry date:	2024-01-01
Number of tests:	24
Number of wells per test:	31+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. 7K2

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*05 Lot (8G3)**

- The product documentation has been updated for new alleles of IMGT 3.38.0.
- The kit resolution focuses on common and well documented (CWD) alleles¹.

¹As described in section Uniquely Identified Alleles.

The DQB1*05 primer set, specificity and interpretation tables have been updated for the HLA-DQB1 alleles described since the previous *Olerup SSP®* DQB1*05 lot was made (**Lot No. 8G3**).

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	Added	-	5'-primer added for the DQB1*05:207 allele.
2	-	Added	3'-primer added for the DQB1*05:218 allele.
4	Added	-	5'-primer added for the DQB1*05:207 allele.
18	-	Added	3'-primer added for the DQB1*06:325 allele..

¹S. J. Mack, P. Cano, J. A. Hollenbach et al.
Common and well-documented HLA alleles: 2012 update to
the CWD catalogue. Tissue Antigens, 2013, 81, 194–203



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Change in revision R01 compared to R00:

1. Primer mix 12 does not amplify the DQB1*05: 84, 05:211, DQB1*03:319, 03:347, DQB1*04:70, DQB1*06:103, 06:284 and 06:350 alleles. This correction has been implemented in the Specificity and Interpretation tables.

Changes in revision R02 compared to R01:

1. Primer mix 8 does not amplify the DQB1*05:27 allele. This correction has been implemented in the Specificity and Interpretation tables.

Changes in revision R03 compared to R02:

1. Primer mix 11 does not amplify the DQB1*03:196 and 06:323 alleles. This correction has been implemented in the Specificity and Interpretation tables.



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Lot No.: **7K2**

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Well **32** contains Negative Control primer pairs, that will amplify a majority of the Olerup SSP[®] HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

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

Length of PCR product	105	200	105	80	75	80	85
5'-primer¹	164	340	440	45	45	43	36
	5'-CAC ^{3'}	5'-Agg ^{3'}	5'-TTA ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-TAC ^{3'}
							36
							5'-TAT ^{3'}
3'-primer²	231	2nd I	507	59	58	57	47
	5'-TgC ^{3'}	5'-AAA ^{3'}	5'-TTg ^{3'}	5'-CTC ^{3'}	5'-ggC ^{3'}	5'-CTC ^{3'}	5'-ACA ^{3'}
							48
							5'-gCA ^{3'}
							48
							5'-gCC ^{3'}
							52
							5'-TgT ^{3'}
A*	+	+	+				
B*	+	+	+				
C*	+	+	+				
DRB1				+	+		
DRB3				+	+		
DRB5				+			
DQB1					+		
DPB1						+	
DQA1							+

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

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



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Lot-specific information

PRODUCT DESCRIPTION

DQB1*05 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DQB1*05:01 to DQB1*05:243 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 32 PCR reactions in a 32 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	NC

The 32 well cut PCR plate is marked with ‘DQB1*05’ in silver gray ink.

Well No. 1 is marked with the Lot No. ‘7K2’.

Wells 1 to 31 – DQB1*05 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 DQB1 alleles, non-DQB1*05 alleles will be amplified by some primer mixes. For further details see Specificity Table.

UNIQUELY IDENTIFIED ALLELES

All the DQB1*05 alleles, i.e. **DQB1*05:01 to DQB1*05:243**, recognized by the HLA Nomenclature Committee in October 2019^{1,2} will be amplified by the primers in the DQB1*05 subtyping kit.

The DQB1*05 kit enables separation of the confirmed DQB1*05 alleles as listed in the IMGT/HLA database 3.26.0. An HLA allele is listed as confirmed by IMGT/HLA if it has been sequenced by more than a single laboratory or from multiple sources. Current allele confirmation status for DQB1*05 alleles is listed below.



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The DQB1*05 also enables identification of many null and alternatively expressed alleles.

The following DQB1*05 alleles can be distinguished by the different sizes of the specific PCR product:

Alleles	Primer mix	Alleles	Primer mix
DQB1*05:12, 05:242	10	DQB1*05:35, 05:117	11
DQB1*05:13, 05:201	11	DQB1*05:45, 05:169, 05:182	11
DQB1*05:16, 05:101	14	DQB1*05:110N, 05:156	27
DQB1*05:20, 05:159	16		

¹DQB1 alleles listed on the IMGT/HLA web page 2019-October-17, release 3.38.0, www.ebi.ac.uk/imgt/hla.

²Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page <http://hla.alleles.org/alleles/deleted.html>.



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Lot No.: 7K2

Lot-specific information

ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
DQB1*05:01:01:01	Confirmed	DQB1*05:03:13	Unconfirmed	DQB1*05:46	Unconfirmed	DQB1*05:94	Unconfirmed
DQB1*05:01:01:02	Unconfirmed	DQB1*05:03:14	Confirmed	DQB1*05:47	Unconfirmed	DQB1*05:95	Unconfirmed
DQB1*05:01:01:03	Confirmed	DQB1*05:03:15	Unconfirmed	DQB1*05:48	Unconfirmed	DQB1*05:96	Unconfirmed
DQB1*05:01:02	Unconfirmed	DQB1*05:03:16	Confirmed	DQB1*05:49	Confirmed	DQB1*05:97	Confirmed
DQB1*05:01:03	Unconfirmed	DQB1*05:03:17	Unconfirmed	DQB1*05:50	Unconfirmed	DQB1*05:98	Unconfirmed
DQB1*05:01:04	Confirmed	DQB1*05:04	Confirmed	DQB1*05:51	Confirmed	DQB1*05:99	Unconfirmed
DQB1*05:01:05	Confirmed	DQB1*05:05:01	Unconfirmed	DQB1*05:52	Confirmed	DQB1*05:100	Unconfirmed
DQB1*05:01:06	Unconfirmed	DQB1*05:05:02	Unconfirmed	DQB1*05:53	Unconfirmed	DQB1*05:101	Confirmed
DQB1*05:01:07	Unconfirmed	DQB1*05:06:01	Unconfirmed	DQB1*05:54	Unconfirmed	DQB1*05:102	Unconfirmed
DQB1*05:01:08	Confirmed	DQB1*05:06:02	Confirmed	DQB1*05:55	Unconfirmed	DQB1*05:103	Confirmed
DQB1*05:01:09	Unconfirmed	DQB1*05:07	Unconfirmed	DQB1*05:56	Unconfirmed	DQB1*05:104	Confirmed
DQB1*05:01:10	Unconfirmed	DQB1*05:08	Unconfirmed	DQB1*05:57	Confirmed	DQB1*05:105	Unconfirmed
DQB1*05:01:11	Unconfirmed	DQB1*05:09	Unconfirmed	DQB1*05:58	Unconfirmed	DQB1*05:106	Confirmed
DQB1*05:01:12	Unconfirmed	DQB1*05:10	Confirmed	DQB1*05:59	Unconfirmed	DQB1*05:107	Unconfirmed
DQB1*05:01:13	Unconfirmed	DQB1*05:11:01	Unconfirmed	DQB1*05:60	Unconfirmed	DQB1*05:108	Unconfirmed
DQB1*05:01:14	Unconfirmed	DQB1*05:11:02	Confirmed	DQB1*05:61	Unconfirmed	DQB1*05:109	Unconfirmed
DQB1*05:01:15	Unconfirmed	DQB1*05:12	Unconfirmed	DQB1*05:62	Unconfirmed	DQB1*05:110N	Unconfirmed
DQB1*05:01:16	Unconfirmed	DQB1*05:13	Unconfirmed	DQB1*05:63	Confirmed	DQB1*05:111	Confirmed
DQB1*05:01:17	Unconfirmed	DQB1*05:14	Unconfirmed	DQB1*05:64	Unconfirmed	DQB1*05:112	Confirmed
DQB1*05:01:18	Unconfirmed	DQB1*05:15	Unconfirmed	DQB1*05:65	Unconfirmed	DQB1*05:113	Unconfirmed
DQB1*05:01:19	Unconfirmed	DQB1*05:16	Unconfirmed	DQB1*05:66:01	Unconfirmed	DQB1*05:114	Unconfirmed
DQB1*05:01:20	Unconfirmed	DQB1*05:17	Unconfirmed	DQB1*05:66:02	Unconfirmed	DQB1*05:115	Unconfirmed
DQB1*05:01:21	Unconfirmed	DQB1*05:18	Confirmed	DQB1*05:67	Unconfirmed	DQB1*05:116	Unconfirmed
DQB1*05:01:22	Unconfirmed	DQB1*05:19	Confirmed	DQB1*05:68	Unconfirmed	DQB1*05:117	Unconfirmed
DQB1*05:02:01	Confirmed	DQB1*05:20	Confirmed	DQB1*05:69	Confirmed	DQB1*05:118	Unconfirmed
DQB1*05:02:02	Unconfirmed	DQB1*05:21	Unconfirmed	DQB1*05:70	Unconfirmed	DQB1*05:119	Unconfirmed
DQB1*05:02:03	Unconfirmed	DQB1*05:22	Unconfirmed	DQB1*05:71	Confirmed	DQB1*05:120	Unconfirmed
DQB1*05:02:04	Unconfirmed	DQB1*05:23	Confirmed	DQB1*05:72	Confirmed	DQB1*05:121	Unconfirmed
DQB1*05:02:05	Unconfirmed	DQB1*05:24	Unconfirmed	DQB1*05:73	Confirmed	DQB1*05:122	Unconfirmed
DQB1*05:02:06	Unconfirmed	DQB1*05:25	Unconfirmed	DQB1*05:74	Unconfirmed	DQB1*05:123	Unconfirmed
DQB1*05:02:07	Confirmed	DQB1*05:26	Confirmed	DQB1*05:75	Confirmed	DQB1*05:124	Unconfirmed
DQB1*05:02:08	Unconfirmed	DQB1*05:27	Confirmed	DQB1*05:76	Unconfirmed	DQB1*05:125	Unconfirmed
DQB1*05:02:09	Unconfirmed	DQB1*05:28	Confirmed	DQB1*05:77	Unconfirmed	DQB1*05:126	Unconfirmed
DQB1*05:02:10	Unconfirmed	DQB1*05:29	Unconfirmed	DQB1*05:78	Unconfirmed	DQB1*05:127	Unconfirmed
DQB1*05:02:11	Confirmed	DQB1*05:30	Unconfirmed	DQB1*05:79	Confirmed	DQB1*05:128N	Unconfirmed
DQB1*05:02:12	Unconfirmed	DQB1*05:31	Unconfirmed	DQB1*05:80	Confirmed	DQB1*05:129	Unconfirmed
DQB1*05:02:13	Unconfirmed	DQB1*05:32	Unconfirmed	DQB1*05:81	Unconfirmed	DQB1*05:130	Unconfirmed
DQB1*05:03:01:01	Confirmed	DQB1*05:33	Unconfirmed	DQB1*05:82	Unconfirmed	DQB1*05:131	Unconfirmed
DQB1*05:03:01:02	Unconfirmed	DQB1*05:34	Confirmed	DQB1*05:83	Unconfirmed		
DQB1*05:03:02	Confirmed	DQB1*05:35	Unconfirmed	DQB1*05:84	Unconfirmed		
DQB1*05:03:03	Unconfirmed	DQB1*05:36	Unconfirmed	DQB1*05:85	Unconfirmed		
DQB1*05:03:04	Unconfirmed	DQB1*05:37	Unconfirmed	DQB1*05:86	Unconfirmed		
DQB1*05:03:05	Unconfirmed	DQB1*05:38	Unconfirmed	DQB1*05:87Q	Unconfirmed		
DQB1*05:03:06	Unconfirmed	DQB1*05:39	Unconfirmed	DQB1*05:88	Confirmed		
DQB1*05:03:07	Unconfirmed	DQB1*05:40	Unconfirmed	DQB1*05:89:01	Unconfirmed		
DQB1*05:03:08	Confirmed	DQB1*05:41N	Unconfirmed	DQB1*05:89:02	Unconfirmed		
DQB1*05:03:09	Unconfirmed	DQB1*05:42	Unconfirmed	DQB1*05:90N	Unconfirmed		
DQB1*05:03:10	Unconfirmed	DQB1*05:43	Confirmed	DQB1*05:91	Unconfirmed		
DQB1*05:03:11	Unconfirmed	DQB1*05:44	Confirmed	DQB1*05:92	Unconfirmed		
DQB1*05:03:12	Unconfirmed	DQB1*05:45	Unconfirmed	DQB1*05:93	Unconfirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2016-October-14, release 3.26.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DQB1*05 homo- and heterozygotes is available upon request.



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Lot No.: **7K2**

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“Instructions for Use” (IFU)

Lot No.: **7K2**

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SPECIFICITY TABLE

DQB1*05 SSP subtyping

Specificities and sizes of the PCR products of the 31+1 primer mixes used for DQB1*05 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DQB1*05 alleles ³	Other amplified DQB1 alleles
1	225 bp	515 bp	*05:01:01:01-05:20, 05:22-05:33, 05:35-05:43:02, 05:45-05:53, 05:55-05:59, 05:61-05:71, 05:74-05:81, 05:84-05:97, 05:99-05:104, 05:106-05:115, 05:117-05:127, 05:129, 05:131-05:174, 05:176-05:217, 05:219-05:243	*06:325
2	135 bp	430 bp	*05:01:01:01-05:01:38, 05:07, 05:11:01-05:12, 05:18, 05:20, 05:22, 05:25, 05:27, 05:29-05:32, 05:44-05:45, 05:48-05:49, 05:51, 05:54-05:55, 05:61-05:63, 05:68-05:76, 05:80-05:81, 05:84, 05:88-05:89:02, 05:92-05:93, 05:95, 05:99, 05:103-05:104, 05:107, 05:110N-05:112, 05:114-05:115, 05:120, 05:122, 05:124, 05:126-05:128N, 05:133, 05:137-05:139, 05:141, 05:144, 05:148, 05:150-05:152, 05:154-05:160, 05:162-05:164, 05:166-05:169, 05:171, 05:173, 05:176-05:177, 05:180, 05:182-05:185N, 05:187-05:188, 05:190, 05:193-05:195, 05:197, 05:215N-05:219, 05:223, 05:225-05:226, 05:228, 05:230, 05:232, 05:234, 05:237, 05:240, 05:242	*06:325
3⁴	120 bp	430 bp	*05:02:01:01-05:02:22, 05:05:01-05:05:02, 05:14, 05:17, 05:19, 05:26, 05:33-05:37, 05:46-05:47, 05:53, 05:57, 05:59, 05:65, 05:79, 05:83, 05:86-05:87Q, 05:90N, 05:94, 05:100, 05:102, 05:106, 05:113, 05:116-05:118, 05:123, 05:125, 05:135-05:136, 05:142, 05:145, 05:153, 05:165, 05:170, 05:172, 05:174-05:175, 05:178-05:179, 05:181, 05:186, 05:189, 05:192, 05:196, 05:198-05:199, 05:222, 05:227, 05:229, 05:231, 05:238-05:239, 05:241	
4⁴	100 bp	515 bp	*05:01:09-05:01:10, 05:02:02, 05:02:18, 05:03:01-01-05:03:13, 05:03:15-05:03:19, 05:03:21-05:03:23, 05:06:01-05:06:02, 05:08-05:10, 05:13, 05:15-05:16, 05:24, 05:28, 05:38-05:42, 05:43:02, 05:50, 05:56, 05:58, 05:60, 05:64, 05:66:01-05:67, 05:78, 05:85, 05:91, 05:96, 05:98, 05:101, 05:108-05:109, 05:119, 05:121, 05:129-05:130, 05:140, 05:143, 05:147, 05:149, 05:161, 05:191, 05:200-05:214,	*03:226, 03:272, 04:28, 06:156, 06:162, 06:169



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“Instructions for Use” (IFU)

Lot No.: **7K2**

Lot-specific information

			05:218, 05:220-05:221, 05:224N, 05:233, 05:235N-05:236N, 05:240, 05:243 *05:04, 05:52, 05:77, 05:132Q, 05:146 *05:10	*03:309
5 ⁴	120 bp 185 bp	430 bp		
6	185 bp	430 bp	*05:05:01-05:05:02, 05:11:01-05:11:02, 05:66:01-05:66:02, 05:240	*06:06 [?] , 06:129, 06:146:01, 06:241
7	180 bp 245 bp 270 bp	430 bp	*05:06:01-05:06:02, 05:07, 05:50 *05:52 *05:112	
8	135 bp 190 bp	430 bp	*05:87Q, 05:132Q *05:09, 05:29	
9	130 bp	430 bp	*05:08, 05:18, 05:36	*06:221, 06:254 ^w , 06:273, 06:326
10 ⁴	115 bp 195 bp	430 bp	*05:23, 05:242 *05:12, 05:71	
11 ⁴	100 bp 150 bp 190 bp	515 bp	*05:13, 05:32, 05:42, 05:45, 05:117, 05:169, 05:201 *05:35, 05:182 *05:96	*04:56 *02:64, 02:79, 03:21, 06:243, 06:255
12 ⁴	120 bp 150 bp 195 bp	430 bp	*05:25 *05:14 *05:41N, 05:90N, 05:96	*02:96N
13	145 bp 180 bp 220 bp	430 bp	*05:40 *05:15, 05:33, 05:49 *05:34, 05:44, 05:128N, 05:130	
14	155 bp 205 bp	430 bp	*05:40, 05:69, 05:101, 05:145, 05:153 *05:16, 05:73, 05:98, 05:116	*06:156, 06:162, 06:169
15 ^{4,5}	65 bp 105 bp 135 bp	430 bp	*05:03:06, 05:03:14, 05:03:20 *05:17 *05:37, 05:104	*03:03:05 *06:250
16 ⁵	145 bp 195 bp	430 bp	*05:20, 05:105 *05:47, 05:159	*02:99, 03:249, 03:312, 06:105, 06:185, 06:225
17 ^{4,7}	125 bp 200 bp	430 bp	*05:25, 05:31, 05:46, 05:108 *05:41N, 05:90N, 05:106	*03:317:01-03:317:02, 06:111, 06:265 *02:96N
18	180 bp 220 bp	430 bp	*05:24 *05:72, 05:80, 05:128N	*06:325
19	195 bp 225 bp	430 bp	*05:28, 05:30 *05:21, 05:60	
20 ⁴	110 bp 210 bp 270 bp	430 bp	*05:32, 05:42, 05:57 *05:106 *05:38, 05:62, 05:119	*04:56 *06:146:01
21	140 bp 165 bp 200 bp 230 bp	430 bp	*05:37, 05:88, 05:104 *05:63 *05:39 *05:26, 05:82	*06:250
22 ⁴	70 bp	430 bp	*05:03:14, 05:03:20, 05:43:01, 05:131	*03:10:02:01-03:10:03, 03:38:02, 03:183, 04:02:07 ^w , 06:01:12, 06:02:43, 06:03:33, 06:51:01, 06:66,



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Lot No.: **7K2**

Lot-specific information

Lot No.: 7K2		Lot-specific information	
			06:96:01, 06:99:01, 06:172, 06:322
23⁴	125 bp	430 bp	*05:19, 05:22, 05:97, 05:143
24	150 bp 185 bp	430 bp	*05:71 *05:73, 05:80, 05:98, 05:116
			*06:28, 06:56, 06:79:01- 06:79:02, 06:89
25⁴	115 bp 180 bp	430 bp	*05:75, 05:111 *05:112
26^{4,6}	85 bp 185 bp	430 bp	*05:103, 05:231 *05:78
27	145 bp 200 bp 280 bp	430 bp	*05:88 *05:110N, 05:236N *05:79, 05:156
			*06:158N
28	135 bp 170 bp	430 bp	*05:56 *05:107
29	135 bp	430 bp	*05:02:01:01-05:02:12, 05:02:14-05:02:22, 05:04-05:05:02, 05:14, 05:17, 05:19, 05:26, 05:33-05:37, 05:46-05:47, 05:52- 05:53, 05:57, 05:59, 05:65, 05:77, 05:79, 05:83, 05:86-05:87Q, 05:90N, 05:94, 05:100, 05:102, 05:106, 05:113, 05:116- 05:118, 05:123, 05:125, 05:132Q, 05:135- 05:136, 05:142, 05:145-05:146, 05:153, 05:165, 05:170, 05:172, 05:174-05:175, 05:178-05:179, 05:181, 05:186, 05:189, 05:192, 05:196, 05:198-05:199, 05:222, 05:227, 05:229, 05:231, 05:238-05:239, 05:241
30^{4,6}	100 bp 145 bp	430 bp	*05:105, 05:111 *05:51
			*03:03:05, 06:02:19, 06:03:08, 06:09:11, 06:13:03, 06:79:02
31⁴	125 bp	430 bp	*05:102
			*02:157, 03:190, 04:42, 06:223
32⁸	-	-	Negative Control

¹Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of DQB1*05 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.

²The 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



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Lot No.: 7K2

Lot-specific information

can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

³For several DQB1 alleles 1st and/or 3rd exon(s) and beyond, as well as intron nucleotide sequences, are not available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. Assumption is made that unknown sequences in these regions are conserved within allelic groups.

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

⁵Primer mixes 15 and 16 may have a tendency of unspecific amplification.

⁶Primer mixes 26 and 30 may give rise to a lower yield of HLA-specific PCR product than the other DQB1*05 primer mixes.

⁷Primer mix 17 may have a tendency in giving rise to primer oligomer formation.

⁸Primer mix 32 contains a negative control, which will amplify a majority of HLA amplicons as well as the amplicons generated by the control primer pairs matching the human growth hormone gene. HLA-specific PCR product sizes range from 75 to 200 base pairs and the PCR product generated by the HGH positive control primer pair is 200 base pairs.

Abbreviations

w: might be weakly amplified.

?: nucleotide sequence information not available for the primer matching sequence.



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Lot No.: **7K2**

Lot-specific information

PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	225	135	120	100	120	185	180	135	130	115	100	120
					185		245	190		195	150	150
							270				190	195
Length of int. pos. control ¹	515	430	430	515	430	430	430	430	430	430	515	430
5'-primer(s) ²	26(173) 5'-ggg 3'	26(173) 5'-ggg 3'	30(184) 5'-gAC 3'	26(173) 5'-gTg 3'	30(184) 5'-gAT 3'	38(210) 5'-gCg 3'	10(126) 5'-CAC 3'	37(205) 5'-Agg 3'	135(501) 5'-gAT 3'	26(173) 5'-ggg 3'	14(136) 5'-gCC 3'	30(185) 5'-ACC 3'
	26(173) 5'-ggg 3'	26(173) 5'-ggg 3'		30(184) 5'-gAC 3'	135(500) 5'-TgA 3'	38(210) 5'-gCA 3'	20(154) 5'-ACA 3'	139(511) 5'-..A 3'			120(454) 5'-Tgg 3'	118(449) 5'-CTA 3'
	26(173) 5'-gTg 3'						39(212) 5'-gCA 3'	139(512) 5'-gAT 3'			133(493) 5'-TTT 3'	120(454) 5'-Tgg 3'
							40(214) 5'-gCC 3'				146(533) 5'-CCg 3'	133(494) 5'-TCA 3'
							40(216) 5'-TTg 3'				151(547) 5'-ACA 3'	
3'-primer(s) ³	87(356) 5'-ggT 3'	57(266) 5'-CAA 3'	57(265) 5'-gCT 3'	47(237) 5'-CgA 3'	57(265) 5'-gCT 3'	86(353) 5'-ACg 3'	87(356) 5'-ggT 3'	86(353) 5'-ACg 3'	163(585) 5'-gTT 3'	51(248) 5'-gCT 3'	32(191) 5'-TAC 3'	57(266) 5'-CAA 3'
	87(356) 5'-ggT 3'	87(356) 5'-ggC 3'	58(270) 5'-TCC 3'	47(237) 5'-CgA 3'	182(642) 5'-ggT 3'			170(604) 5'-gAC 3'	167(596) 5'-CAT 3'	74(316) 5'-Cgg 3'	170(604) 5'-gAC 3'	170(604) 5'-gAC 3'
				48(240) 5'-gCg 3'						78(328) 5'-CAA 3'		
				50(246) 5'-gTg 3'								
Well No.	1	2	3	4	5	6	7	8	9	10	11	12



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Lot-specific information

Well No.	13	14	15	16	17	18	19	20	21	22	23	24
Length of spec.	145	155	65	145	125	180	195	110	140	70	125	150
PCR product	180	205	105	195	200	220	225	210	165			185
			220					270	200			
			135						230			
Length of int.	430	430	430	430	430	430	430	430	430	430	430	430
pos. control ¹												
5'-primer(s) ²	30(184) 5'-gAC 3'	30(184) 5'-gAC 3'	49(243) 5'-gCA 3'	20(154) 5'-ACC 3'	30(185) 5'-ACC 3'	30(184) 5'-gAC 3'	25(170) 5'-gCA 3'	9(122) 5'-gTT 3'	24(166) 5'-gCT 3'	47(237) 5'-TAC 3'	26(173) 5'-ggg 3'	38(209) 5'-CgT 3'
			135(501) 5'-gAT 3'	26(173) 5'-ggA 3'	113(435) 5'-AAA 3'		25(170) 5'-gCT 3'	113(435) 5'-AAA 3'	26(172) 5'-ggA 3'			
				135(500) 5'-TgA 3'	118(449) 5'-CTA 3'		31(187) 5'-ACC 3'	146(533) 5'-CCg 3'	33(195) 5'-AAg 3'			
					141(517) 5'-CCA 3'		38(208) 5'-ACA 3'	147(535) 5'-CCg 3'	45(230) 5'-ggA 3'			
									52(251) 5'-gCT 3'			
									135(501) 5'-gAT 3'			
3'-primer(s) ³	65(290) 5'-CCC 3'	65(290) 5'-CCC 3'	57(266) 5'-CgT 3'	57(266) 5'-CAA 3'	57(266) 5'-CAA 3'	76(323) 5'-TgC 3'	86(353) 5'-ACg 3'	86(353) 5'-ACg 3'	87(356) 5'-ggT 3'	57(265) 5'-gTC 3'	49(242) 5'-CCT 3'	74(316) 5'-Cgg 3'
	72(311) 5'-CCT 3'	70(304) 5'-CCT 3'	157(566) 5'-ggg 3'	186(652) 5'-CAT 3'	170(604) 5'-gAC 3'	86(353) 5'-ACC 3'		170(604) 5'-gAC 3'	167(595) 5'-ACA 3'		54(257) 5'-gCT 3'	86(353) 5'-ACC 3'
	74(317) 5'-CCA 3'	71(307) 5'-ggA 3'	167(595) 5'-ACA 3'			88(358) 5'-gCC 3'					58(268) 5'-ggT 3'	86(353) 5'-ACg 3'
	80(335) 5'-gTT 3'	82(341) 5'-AgC 3'				92(370) 5'-CTA 3'						
	89(361) 5'-CCT 3'	87(356) 5'-ggA 3'										
	92(370) 5'-CTA 3'											
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

Well No.	25	26	27	28	29	30	31
Length of spec.	115	85	145	135	135	100	125
PCR product	180	185	200	170		145	
			280				
Length of int.	430	430	430	430	430	430	430
pos. control ¹							
5'-primer(s) ²	10(126) 5'-CAC 3'	116(442) 5'-TgA 3'	8(118) 5'-TCA 3'	135(500) 5'-TgA 3'	26(173) 5'-ggg 3'	14(136) 5'-gCA 3'	102(400) 5'-TCT 3'
	32(190) 5'-TCC 3'		34(196) 5'-ACT 3'			26(173) 5'-ggA 3'	
	33(193) 5'-ATT 3'		52(251) 5'-gCT 3'			33(193) 5'-ATT 3'	
3'-primer(s) ³	57(266) 5'-CAA 3'	130(485) 5'-ACT 3'	87(356) 5'-ggT 3'	166(594) 5'-CgA 3'	57(265) 5'-gCT 3'	49(241) 5'-TgC 3'	130(484) 5'-CCA 3'
		164(586) 5'-AgA 3'		177(627) 5'-ggT 3'			
Well No.	25	26	27	28	29	30	31

¹The 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.



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Lot-specific information

²The nucleotide position matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

³The nucleotide position matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.



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Lot-specific information

CELL LINE VALIDATION SHEET																				
DQB1*05 SSP subtyping kit																				
				Well ²																
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
				Prod. No.	202014701	202014702	201560003	202014704	201560005	201560006	201779507	201779508	201560009	201560010	201779511	201779512	201779513	201779514	201560015	202014716
IHWC cell line ¹			DQB1																	
1	9001	SA	*05:01		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280	LK707	*06:01	*02:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011	E4181324	*06:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275	GU373	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009	KAS011	*05:02		+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353	SM	*03:02	*06:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	9020	QBL	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025	DEU	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026	YAR	*03:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107	LKT3	*04:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051	PITOUT	*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052	DBB	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004	JESTHOM	*05:01		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071	OLGA	*04:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075	DKB	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037	SWEIG007	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282	CTM3953540	*02:01	*06:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257	32367	*06:02	*02:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038	BM16	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059	SLE005	*06:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064	AMALA	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056	KOSE	*05:03	*06:04	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
23	9124	IHL	*05:03	*06:01	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
24	9035	JBUSH	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049	IBW9	*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285	WT49	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191	CH1007	*04:01	*05:01	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320	BEL5GB	*02:02	*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050	MOU	*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021	RSH	*04:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019	DUCAF	*02:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297	HAG	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098	MT14B	*03:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104	DHIF	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302	SSTO	*03:05		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024	KT17	*03:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065	HHKB	*06:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099	LZL	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315	CML	*02:01	*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134	WHONP199	*02:02	*03:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055	H0301	*06:09		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066	TAB089	*06:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076	T7526	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057	TEM	*05:03		+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
45	9239	SHJO	*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013	SCHU	*06:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045	TUBO	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303	TER-ND	*05:01		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-



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Lot-specific information

CELL LINE VALIDATION SHEET																			
DQB1*05 SSP subtyping kit																			
				Prod. No.	Well ²														
					17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
					201779517	202014718	201779519	201779520	201779521	201779522	201779523	201779524	202014725	201779526	201779527	201779528	201779529	201779530	201779531
	IHWC cell line ¹	DQB1																	
1	9001 SA	*05:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707	*06:01	*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324	*06:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	9275 GU373	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*05:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	9353 SM	*03:02	*06:01		-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
7	9020 QBL	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	9025 DEU	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*03:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	9107 LKT3	*04:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*03:03			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM	*05:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*04:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*03:03			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540	*02:01	*06:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	9257 32367	*06:02	*02:02		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*06:04			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*05:03	*06:04		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*05:03	*06:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	9035 JBUSH	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*04:01	*05:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB	*02:02	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*04:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*02:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*03:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*03:05			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*03:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	9065 HHKB	*06:03			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML	*02:01	*03:01		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	9134 WHONP199	*02:02	*03:03		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*06:09			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*06:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*03:03			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*05:03			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	9239 SHJO	*02:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU	*06:02			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*03:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	9303 TER-ND	*05:01			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



101.211-24 – including *Taq* polymerase
101.211-24u – without *Taq* polymerase

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Lot No.: 7K2

Lot-specific information

¹The 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.

²The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs

No DNAs carrying the alleles to be amplified by primer solutions 5 to 28, 30 and 31 were available. The specificities of the primers in primer solutions 5, 6, 8, 11, 14 to 16, 18, 20 to 22, 24, 26, 27 and 30 were tested by separately adding one or two 5'-primers, respectively one, two or three 3'-primers. In primer solutions 7, 12, 17, 19 and 25 it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 9, 10, 13, 23, 28 and 31 it was only possible to test the 5'-primer, the 3'-primers were not possible to test. In primer solutions 1, 2, 4, 8, 11, 16, 20, 21, 27 and 30 one or more 5'-primers were not possible to test, and in primer solutions 1 to 5, 11, 14 to 16, 18, 21, 24 and 26 one or more 3'-primers were not possible to test. In addition, one 3'-primer in primer solution 4 was tested by adding separately one additional 5'-primer.



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101.211-24u – without *Taq* polymerase

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Lot-specific information

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