

University Clinic Heidelberg  
 Transplantation Immunology  
 Im Neuenheimer Feld 305  
 69120 Heidelberg - Germany  
 Phone: +49 6221 564013  
 Fax: +49 6221 564200  
 www.ctstransplant.org

Manual No.	28
Revision	July 17, 2017
Product No.	128
Lot No.	DQB11-2 DQA09-0


**CTS** Collaborative Transplant Study

## WORKING INSTRUCTION

### HLA- DQB1\*/-DQA1\* low resolution CTS-PCR-SSP TRAY KIT

#### LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following product:

Product No.	Description
128	HLA-DQB1*/-DQA1* low resolution CTS-PCR-SSP Tray Kit 

#### 1. Main differences

- **Attention:**  
 All CTS-PCR-SSP Kits (for both, HLA class I and HLA class II typing) can be used with the new "Mastermix SSP" as PCR buffer. There is no change in the Mastermix volume to be used for setting up the PCR reaction. Taq polymerase is also required as previously.
- **Between Lot DQB11-2 (the current lot) and Lot DQB11-1:**  
 The kit was updated to cover new alleles included in the IMGT/HLA Database of January 2017. Deleted and renamed alleles were taken into consideration.
- **Between Lot DQA09-0 (the current lot) and Lot DQA08-2:**  
 The kit was updated to cover new alleles included in the IMGT/HLA Database of January 2017. Deleted and renamed alleles were taken into consideration.

#### 2. Introduction

- Intended use: This kit reveals a low/intermediate resolution typing of HLA-DRB1\* (besides HLA-DRB3\*/4\*/5\* low resolution) and -DQB1\* by the PCR-SSP method.
- Allele coverage: IMGT/HLA Sequence Database Release 3.27.0, January 2017 for HLA-DQB1 and -DQA1, except:  
 HLA-DQB1\*02:25/02:35/02:40/02:72, DQB1\*03:17/02/03:72/03:100, DQB1\*04:02:02/04:02:08/04:31, DQB1\*05:01:14/05:03:10/05:21/05:60/05:72/05:73/05:82/05:98/05:105/05:116 and DQB1\*06:02:08/06:03:19/06:48/06:51:02/06:153/06:167
- These alleles are considered to be rare and can be detected e. g. by sequencing with our CTS-SEQUENCE Kits (you may contact us for further information).
- This manual is only valid for **Lot No. DQB11-2 DQA09-0**
- This manual should be used together with the Main Manual (General Information) which is the 'Working instruction for the CTS-PCR-SSP **TRAY and MINITRAY KITS**' (Manual No. 100A).

### 3. Content

1. Main differences .....	1
2. Introduction .....	1
3. Content.....	2
4. Kit Composition.....	3
5. Materials, Reagents and Equipment not supplied .....	3
6. Sample Requirements, PCR and Gel Electrophoresis.....	3
7. Result Evaluation.....	3
8. Interpretation Hints .....	3
9. Special notes .....	3
10. Troubleshooting .....	3
11. Precaution.....	3
12. Contact.....	4
13. Appendix .....	5
Table 1: Sizes of the PCR products and allele specificities of each HLA-DQB1* CTS-PCR-SSP primer mix (Lot-No DQB11-2) based on IMGT/HLA Sequence Database Release 3.27.0, January 2017 .....	5
Table 2: Sizes of the PCR products and allele specificities of each HLA-DQA1* CTS-PCR-SSP primer mix (Lot-No DQA09-0) based on IMGT/HLA Sequence Database Release 3.27.0, January 2017 .....	9
Table 3: Amplification patterns of HLA-DQB1* alleles detected by the HLA-DQB1* CTS-PCR-SSP primer mixes (Lot No. DQB11-2) based on IMGT/HLA Sequence Database Release 3.27.0, January 2017 .....	10
Table 4: Amplification patterns for all detectable HLA-DQA1* specificities (Lot-No DQA09-0) based on IMGT/HLA Sequence Database Release 3.27.0, January 2017 .....	13

#### 4. Kit Composition

- Number of PCR primer mixes per test: 23, of which:
  - 12 mixes for HLA-DQB1 typing (12 allele-specific mixes)
  - 11 mixes for HLA-DQA1 typing (10 allele-specific mixes + 1 negative control mix)
  - Please note: Wells (positions on tray) A3, A6, A9 and A12 are empty
- Number of tests per tray: 4
- Number of trays per kit: 10
- The primer mixes are aliquoted and dried in thin-walled, yellow PCR-Trays.
- PCR buffer: 3.0 ml of Mastermix SSP (without Taq polymerase).

For storage condition, please refer to Section 1 of the 'Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No. 100A) supplied along with this product.

#### 5. Materials, Reagents and Equipment not supplied

Please refer to Section 2 of the 'Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No. 100A) supplied along with this product.

#### 6. Sample Requirements, PCR and Gel Electrophoresis

Please refer to Section 3 to 6 of the 'Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No. 100A) supplied along with this product.

#### 7. Result Evaluation

- Check the approximate size of the PCR product against the Primer Mix Specificity Tables (Appendix / Table 1 and 2) to confirm the correct product size.
- Use the Amplification Pattern Tables (Appendix / Table 3 and 4) to make the allele assignments or use the SCORE Software ([www.IHWG.org](http://www.IHWG.org)) for detailed result interpretation.

#### 8. Interpretation Hints

- The quality and quantity of DNA as well as of the Taq polymerase are extremely crucial factors. If your bands are too weak, you might try to adjust these two factors until you obtain optimal results.
- Please also refer to Section 7 of the 'Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No. 100A) supplied along with this product.

#### 9. Special notes

- **HLA-DQB1\* locus:**  
Mix 6 and 7 mainly detect HLA-DQB1\*03 alleles which belong to the serological group of HLA-DQ7(3). Mix 8 and 9 react positively with HLA-DQB1\*03 alleles of the HLA-DQ8(3) serological group, whereas mix 10 amplifies HLA-DQB1\*03 alleles which can serologically be defined as HLA-DQ9(3). Some other less common alleles are amplified by these mixes in addition.
- **HLA-DQA1\* locus:**  
HLA-DQA1\*05:04 generates two PCR fragments with Mix 9.

#### 10. Troubleshooting

Please refer to Section 8 of the 'Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No. 100A) supplied along with this product.

#### 11. Precaution

Please refer to Material Safety Data Sheet for the CTS-PCR-SSP TRAY and MINITRAY KITS (Manual No. 100B) supplied along with this product.

## 12. Contact

If you have any particular questions concerning this kit, which are not answered in this or the Main Manual, please do not hesitate to contact me or my coworkers at:

Phone: +49 6221 564013

Fax: +49 6221 564200

E-mail: [hien.tran@med.uni-heidelberg.de](mailto:hien.tran@med.uni-heidelberg.de)

Hien Tran, M.D.

### 13. Appendix

**Table 1:** Sizes of the PCR products and allele specificities of each **HLA-DQB1\*** CTS-PCR-SSP primer mix (**Lot-No DQB11-2**) based on IMGT/HLA Sequence Database Release 3.27.0, January 2017

Position				Mix	Allele	Serology	Size
H1	H4	H7	H10	Mix 1	DQB1*04:10w, DQB1*05:01:01:01-05:01:13/05:01:15/05:01:16w/05:01:17-05:01:18/05:01:19w/05:01:20-05:03:09/05:03:11-05:20/05:22-05:25/05:26w/05:27-05:33/05:34w/05:35-05:43:02/05:44w/05:45-05:59/05:61-05:71/05:74-05:81/05:83-05:97/05:99-05:104/05:106-05:112/05:113w/05:114-05:115/05:117-05:127/05:128Nw/05:129/05:130w/05:131-05:143, DQB1*06:23/06:156/06:162/06:169	-, DQ5(1), Null	See below
					DQB1*04:10w, DQB1*05:03:02/05:43:02, DQB1*06:23/06:156/06:162/06:169	-, DQ5(1)	135 bp
					DQB1*05:01:01:01-05:01:13/05:01:15/05:01:16w/05:01:17-05:01:18/05:01:19w/05:01:20-05:03:09/05:03:11-05:20/05:22-05:25/05:26w/05:27-05:33/05:34w/05:35-05:43:02/05:44w/05:45-05:59/05:61-05:71/05:74-05:81/05:83-05:97/05:99-05:104/05:106-05:112/05:113w/05:114-05:115/05:117-05:127/05:128Nw/05:129/05:130w/05:131-05:143	DQ5(1), -, Null	225 bp
G1	G4	G7	G10	Mix 2	DQB1*03:194, DQB1*06:01:01-06:01:15/06:02:07/06:03:01-06:03:06/06:03:08-06:03:18/06:03:20-06:03:26/06:08:01-06:08:03/06:11:02-06:12/06:14:01/06:14:03/06:17/06:21/06:26N/06:28/06:30-06:31/06:35/06:40-06:45/06:53:01-06:57/06:59-06:65/06:67/06:82/06:87/06:90-06:92/06:98-06:105/06:108/06:110/06:120/06:128/06:132-06:134/06:140-06:145/06:148/06:149w/06:154/06:157/06:165/06:168/06:170/06:177/06:181/06:184-06:185/06:187/06:190:01-06:191/06:194-06:196/06:199/06:203/06:205/06:209-06:210/06:214/06:218/06:221-06:223/06:229-06:230	-, DQ6(1), DQ1, Null	See below
					DQB1*06:01:01-06:01:15/06:35/06:43/06:45/06:53:01-06:57/06:98-06:105/06:108/06:120/06:132/06:140/06:142/06:157/06:168/06:177/06:181/06:194/06:205/06:209/06:214/06:229	DQ6(1), -, Null	160 bp
					DQB1*03:194, DQB1*06:02:07/06:03:01-06:03:06/06:03:08-06:03:18/06:03:20-06:03:26/06:08:01-06:08:03/06:11:02-06:12/06:14:01/06:14:03/06:17/06:21/06:26N/06:28/06:30-06:31/06:40-06:42/06:44/06:59-06:65/06:67/06:82/06:87/06:90-06:92/06:110/06:128/06:133-06:134/06:141/06:143-06:145/06:148/06:149w/06:154/06:165/06:170/06:184-06:185/06:187/06:190:01-06:191/06:195-06:196/06:199/06:203/06:210/06:218/06:221-06:223/06:230	-, DQ6(1), DQ1, Null	170 bp

Position				Mix	Allele	Serology	Size
F1	F4	F7	F10	Mix 3	DQB1*03:08/03:137/03:228, DQB1*06:02:01:01-06:02:07/06:02:09-06:03:03/06:03:05w/06:03:06-06:03:07/06:03:11-06:03:13/06:03:15/06:03:17-06:03:18/06:03:20-06:03:25/06:08:01/06:10-06:11:03/06:13:01-06:14:02/06:16/06:19:01-06:20/06:23-06:24/06:26N/06:28-06:31/06:33/06:40-06:41/06:44/06:46-06:47/06:49-06:50/06:60-06:63/06:65w/06:67-06:68/06:70-06:84/06:87/06:90/06:92/06:95-06:97/06:106-06:107/06:109-06:117/06:119/06:122-06:128/06:130-06:131/06:133-06:134/06:136-06:138/06:141/06:143-06:144N/06:146:01-06:148/06:150-06:152/06:154/06:156/06:159/06:161-06:163/06:165-06:166/06:169-06:170/06:173-06:176/06:178-06:179N/06:182-06:185/06:187-06:188/06:191-06:192/06:195/06:197-06:200/06:201w/06:203/06:206:01-06:206:02/06:208/06:210-06:211/06:213/06:215-06:216N/06:218-06:219/06:221-06:228/06:230	-, DQ6(1), DQ1, Null	See below
					DQB1*03:08/03:137/03:228, DQB1*06:02:01:01-06:02:06/06:02:09-06:02:26/06:03:07/06:10-06:11:01/06:13:01-06:13:02/06:14:02/06:16/06:19:01-06:20/06:24/06:29/06:33/06:46-06:47/06:49-06:50/06:68/06:70-06:81/06:83-06:84/06:95-06:97/06:106-06:107/06:109/06:111-06:117/06:119/06:122-06:127/06:130-06:131/06:136-06:138/06:146:01-06:147/06:150-06:152/06:159/06:161/06:163/06:166/06:173-06:176/06:178-06:179N/06:182-06:183/06:188/06:192/06:197-06:198/06:200/06:201w/06:206:01-06:206:02/06:208/06:211/06:213/06:215-06:216N/06:219/06:224-06:228	-, DQ6(1), DQ1, Null	165 bp
					DQB1*06:02:01:01-06:02:04/06:02:06-06:02:07/06:02:09-06:02:15/06:02:17-06:02:18/06:02:20-06:03:03/06:03:05w/06:03:06-06:03:07/06:03:11-06:03:13/06:03:15/06:03:17-06:03:18/06:03:20-06:03:25/06:08:01/06:10-06:11:03/06:13:01/06:14:01-06:14:02/06:16/06:19:02-06:20/06:23-06:24/06:26N/06:28-06:31/06:33/06:40-06:41/06:44/06:46-06:47/06:49-06:50/06:60-06:63/06:65w/06:67-06:68/06:70-06:79:01/06:80-06:84/06:87/06:90/06:92/06:95-06:97/06:106-06:107/06:109-06:113/06:115-06:117/06:122/06:124-06:128/06:130-06:131/06:133-06:134/06:136-06:137/06:141/06:143-06:144N/06:146:01-06:148/06:151/06:154/06:156/06:159/06:161-06:163/06:165-06:166/06:169-06:170/06:173-06:176/06:178-06:179N/06:182-06:185/06:187-06:188/06:191-06:192/06:195/06:197-06:200/06:201w/06:203/06:206:01/06:208/06:210-06:211/06:213/06:216N/06:218-06:219/06:221-06:228/06:230	DQ6(1), -, DQ1, Null	105 bp
E1	E4	E7	E10	Mix 4	DQB1*06:04:01-06:07:02/06:09:01:01-06:09:03/06:09:05/06:09:06w/06:18:01-06:18:02/06:22:02/06:25/06:27:01-06:27:02/06:32:01-06:32:02/06:34/06:36/06:38-06:39/06:52/06:58/06:66/06:69/06:85-06:86/06:88-06:89/06:93-06:94/06:118:01-06:118:02/06:121w/06:129/06:135/06:142/06:155/06:158N/06:160/06:164/06:168/06:171-06:172/06:180/06:186/06:189/06:193N/06:202/06:204/06:207/06:212w/06:217	DQ6(1), -, Null	170 bp
D1	D4	D7	D10	Mix 5	DQB1*02:01:01-02:01:23/02:01:24w/02:02:01:01-02:24/02:26-02:34/02:36/02:37w/02:38-02:39/02:41-02:45/02:46w-02:47w/02:48-02:71/02:73-02:88	DQ2, -, Null	200 bp

Position				Mix	Allele	Serology	Size
C1	C4	C7	C10	Mix 6	DQB1*03:01:01:01-03:01:37/03:04:01-03:04:03/03:09-03:10:02/03:13-03:14:02/03:16/03:19:01-03:19:02/03:21-03:24/03:27-03:29/03:35/03:36w/03:42/03:44/03:46-03:60/03:69/03:71/03:73/03:75-03:77/03:80/03:82-03:84N/03:92-03:94/03:101-03:103/03:108-03:109/03:114-03:116/03:118N-03:121/03:122w/03:127-03:131/03:133-03:135/03:138-03:140/03:142-03:144/03:147/03:150/03:151w/03:152/03:154/03:157-03:160/03:162-03:167/03:169-03:170/03:171w/03:172-03:173/03:180/03:182-03:183/03:186-03:188/03:191-03:198/03:201-03:202/03:206-03:208/03:216-03:219/03:231-03:232/03:235-03:236/03:241-03:243/03:246/03:252-03:256, DQB1*04:10, DQB1*05:03:02?, DQB1*06:35/06:53:01-06:53:02	DQ7(3), -, DQ3, Null, DQ5(1)	See below
					DQB1*03:01:01:01-03:01:37/03:04:01-03:04:03/03:09/03:13/03:16/03:19:01-03:19:02/03:21-03:22/03:24/03:27-03:29/03:35/03:36w/03:42/03:44/03:46-03:60/03:69/03:71/03:73/03:75-03:77/03:80/03:82-03:84N/03:92-03:94/03:101-03:103/03:108-03:109/03:114-03:116/03:118N-03:121/03:122w/03:127/03:129-03:131/03:133-03:135/03:139-03:140/03:142-03:144/03:147/03:150/03:151w/03:152/03:154/03:157-03:160/03:162-03:167/03:169-03:170/03:171w/03:172-03:173/03:182/03:186-03:188/03:191-03:194/03:196-03:198/03:201-03:202/03:206-03:208/03:216/03:218-03:219/03:231-03:232/03:235-03:236/03:241-03:243/03:246/03:252-03:256, DQB1*06:35/06:53:01-06:53:02	DQ7(3), -, Null	100 bp
					DQB1*03:01:01:01-03:01:37/03:04:01-03:04:03/03:09-03:10:02/03:13-03:14:02/03:16/03:19:01-03:19:02/03:21-03:22/03:24/03:27-03:29/03:35/03:36w/03:42/03:44/03:46-03:60/03:69/03:71/03:73/03:75-03:77/03:80/03:82-03:84N/03:92-03:94/03:101-03:103/03:108-03:109/03:114-03:116/03:118N-03:121/03:122w/03:127-03:131/03:133-03:135/03:138-03:140/03:142-03:144/03:147/03:150/03:151w/03:152/03:154/03:157-03:160/03:162-03:167/03:169-03:170/03:171w/03:172-03:173/03:180/03:182-03:183/03:186-03:188/03:191-03:198/03:201-03:202/03:206-03:207/03:216/03:218-03:219/03:231-03:232/03:235-03:236/03:241-03:243/03:246/03:252-03:256	DQ7(3), -, DQ3, Null	215 bp
					DQB1*03:23:01-03:23:02/03:217, DQB1*04:10, DQB1*05:03:02?	-, DQ5(1)	120 bp
B1	B4	B7	B10	Mix 7	DQB1*03:04:01-03:04:03/03:14:01-03:14:02/03:70/03:80/03:179w	DQ7(3), -	175 bp
A1	A4	A7	A10	Mix 8	DQB1*03:02:01:01-03:02:09/03:02:11-03:02:13/03:02:14w/03:02:15/03:02:17-03:02:24/03:07-03:08/03:11/03:32/03:37/03:45/03:62-03:64/03:66N-03:68/03:70/03:81/03:85/03:106-03:107/03:125/03:146/03:153/03:161/03:174-03:175/03:178-03:179/03:184-03:185/03:189-03:190/03:199/03:203-03:205/03:210-03:211/03:213N-03:215/03:220-03:221/03:223-03:224/03:228-03:229/03:233/03:237N/03:240/03:245/03:247/03:251, DQB1*06:29/06:37/06:123/06:139	DQ8(3), -, Null	See below
					DQB1*03:02:01:01-03:02:09/03:02:11-03:02:13/03:02:14w/03:02:15/03:02:17-03:02:24/03:07-03:08/03:11/03:32/03:37/03:45/03:62-03:64/03:66N-03:68/03:70/03:81/03:85/03:106-03:107/03:125/03:146/03:153/03:161/03:174-03:175/03:178-03:179/03:184-03:185/03:189-03:190/03:199/03:203-03:205/03:210-03:211/03:213N-03:215/03:220-03:221/03:223-03:224/03:228-03:229/03:233/03:237N/03:240/03:245/03:247/03:251, DQB1*06:29/06:123/06:139	DQ8(3), -, Null	130 bp
					DQB1*06:37	-	145 bp
H2	H5	H8	H11	Mix 9	DQB1*03:05:01-03:05:04/03:61/03:132/03:181/03:226/03:250	DQ8(3), -	135 bp

Position				Mix	Allele	Serology	Size
G2	G5	G8	G11	Mix 10	<b>DQB1*03:03:02:01-03:03:13/03:06/03:12/03:15/03:20/03:25:01-03:26/03:30-03:31/03:33-03:34/03:38-03:41/03:43/03:65/03:74/03:78w/03:79/03:86-03:91Q/03:95N-03:99Q/03:104-03:105/03:111-03:113/03:117/03:123-03:124/03:126/03:136-03:137/03:141/03:145/03:155-03:156/03:168/03:176-03:177/03:200/03:209/03:212/03:222/03:227/03:230/03:234/03:238-03:239/03:248-03:249, DQB1*04:03:01-04:03:02, DQB1*06:09:04/06:15:01/06:15:02w/06:22:01/06:22:03/06:46/06:51:01/06:96/06:119</b>	DQ9(3), -, DQ3, Null	See below
					DQB1*03:03:02:01-03:03:13/03:06/03:12/03:15/03:20/03:25:01-03:26/03:30-03:31/03:33-03:34/03:38-03:41/03:43/03:65/03:74/03:78w/03:79/03:86-03:91Q/03:95N-03:99Q/03:104-03:105/03:111-03:113/03:117/03:123-03:124/03:126/03:136-03:137/03:141/03:145/03:155-03:156/03:168/03:176-03:177/03:200/03:209/03:212/03:222/03:227/03:230/03:234/03:238-03:239/03:248-03:249, DQB1*04:03:01-04:03:02, DQB1*06:51:01/06:96	DQ9(3), -, DQ3, Null	130 bp
					DQB1*06:09:04/06:15:01/06:15:02w/06:22:01/06:22:03/06:46/06:119	-	215 bp
F2	F5	F8	F11	Mix 11	DQB1*03:01:01:01-03:01:01:05/03:01:03-03:01:05/03:01:06w/03:01:07-03:02:02/03:02:04w/03:02:05-03:02:12/03:02:13w/03:02:14-03:02:22/03:02:24-03:03:02:04/03:03:04-03:04:03/03:05:03-03:05:04/03:07-03:17:01/03:18-03:19:02/03:21-03:22/03:23:02-03:24/03:25:02-03:36/03:38-03:53/03:54w/03:55-03:60/03:62-03:71/03:74/03:76-03:99Q/03:101-03:111/03:113-03:117/03:119-03:128/03:130-03:131/03:133-03:135/03:137-03:155/03:157-03:161/03:163-03:174/03:176-03:180/03:182/03:184-03:203/03:204w/03:205-03:222/03:224-03:225/03:227-03:236/03:239-03:249/03:251/03:253-03:256, DQB1*05:11:01, DQB1*06:02:02/06:03:02/06:04:08	DQ7(3), -, DQ8(3), DQ9(3), Null, DQ6(1)	160 bp
E2	E5	E8	E11	Mix 12	DQB1*03:132, DQB1*04:01:01-04:02:01:03/04:02:03-04:02:07/04:02:09-04:03:01/04:04-04:30/04:32-04:39	-, DQ4, Null	210 bp

**Amplification control (internal positive control):** 440 base pairs (bp)

**w** = weak

**?** = nucleotide sequence information not available for the primer matching sequence

**Bold:** mixes which result in PCR fragments of different sizes (the specificities are first indicated all in one row, then split into several groups in the subsequent rows depending on the fragment size)

**Mix 6, HLA-DQB1\*03:10:** a rare allele according to Mack SJ et al., Tissue Antigens 2013, serological equivalent is controversially discussed (DQ3, DQ7 or DQ8).



**Table 2:** Sizes of the PCR products and allele specificities of each **HLA-DQA1\*** CTS-PCR-SSP primer mix (**Lot-No DQA09-0**) based on IMGT/HLA Sequence Database Release 3.27.0, January 2017

Position				Mix	Allele	Serology	Size
D2	D5	D8	D11	Mix 1	DQA1*01:01:01:01-01:01:03/01:04:01:01-01:05:02/01:07Q/01:12	-	145 bp
C2	C5	C8	C11	Mix 2	DQA1*01:02:01:01-01:03:01:06/01:06/01:08-01:11/01:13-01:15N	-, Null	145 bp
B2	B5	B8	B11	Mix 3	DQA1*01:01:01:01-01:02:04/01:04:01:01-01:09/01:11-01:13	-	170 bp
A2	A5	A8	A11	Mix 4	DQA1*01:03:01:01-01:03:01:06/01:10/01:14-01:15N	-, Null	170 bp
H3	H6	H9	H12	Mix 5	DQA1*01:04:01:01-01:05:02/01:06?/01:07Q/01:08?-01:09?/01:12?-01:13?/01:15N?, DQA1*03:01:03?, DQA1*04:03N?-04:04?, DQA1*05:02?/05:04?/05:10?, DQA1*06:01:02?-06:02?	-, Null	200 bp
G3	G6	G9	G12	Mix 6	DQA1*02:01:01:01-02:01:01:02	-	105 bp
F3	F6	F9	F12	Mix 7	DQA1*03:01:01/03:01:03-03:03:02	-	130 bp
E3	E6	E9	E12	Mix 8	DQA1*04:01:01-04:04	-, Null	215 bp
D3	D6	D9	D12	Mix 9	<b>DQA1*05:01:01:01-05:09/05:10w/05:11</b>	-	<b>see below</b>
					DQA1*05:04	-	205 bp
					DQA1*05:01:01:01-05:09/05:10w/05:11	-	190 bp
C3	C6	C9	C12	Mix 10	DQA1*06:01:01-06:02	-	105 bp
B3	B6	B9	B12	Mix 11	Negative Control	-	none (440 bp)

**Amplification control (internal positive control):** 440 base pairs (bp)

**Positions:** A3, A6, A9 and A12 are empty wells.

**w** = weak

**?** = nucleotide sequence information not available for the primer matching sequence

**Bold:** mixes which result in PCR fragments of different sizes (the specificities are first indicated all in one row, then split into several groups in the subsequent rows depending on the fragment size)

**Table 3:** Amplification patterns of **HLA-DQB1\*** alleles detected by the HLA-DQB1\* CTS-PCR-SSP primer mixes (**Lot No. DQB11-2**) based on IMGT/HLA Sequence Database Release 3.27.0, January 2017

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12
DQB1*02:01:01-02:01:23/02:02:01-01-02:24/02:26-02:34/02:36/02:38-02:39/02:41-02:45/02:48-02:71/02:73-02:88	DQ2, -, Null					5							
DQB1*02:01:24/02:37/02:46-02:47	-					w							
DQB1*03:01:01-01-03:01:01:05/03:01:03-03:01:05/03:01:07-03:01:37/03:09-03:10:02/03:13/03:16/03:19-01-03:19:02/03:21-03:22/03:23-02-03:24/03:27-03:29/03:35/03:42/03:44/03:46-03:53/03:55-03:60/03:69/03:71/03:76-03:77/03:82-03:84N/03:92-03:94/03:101-03:103/03:108-03:109/03:114-03:116/03:119-03:121/03:127-03:128/03:130-03:131/03:133-03:135/03:138-03:140/03:142-03:144/03:147/03:150/03:152/03:154/03:157-03:160/03:163-03:167/03:169-03:170/03:172-03:173/03:180/03:182/03:186-03:188/03:191-03:193/03:195-03:198/03:201-03:202/03:206-03:208/03:216-03:219/03:231-03:232/03:235-03:236/03:241-03:243/03:246/03:253-03:256	DQ7(3), -, DQ8(3), Null						6					11	
DQB1*03:01:02/03:23-01/03:73/03:75/03:118N/03:129/03:162/03:183/03:252	DQ7(3), -, Null						6						
DQB1*03:01:06/03:54	-						6					w	
DQB1*03:02:01:01-03:02:02/03:02:05-03:02:09/03:02:11-03:02:12/03:02:15/03:02:17-03:02:22/03:02:24/03:07/03:11/03:32/03:45/03:62-03:64/03:66N-03:68/03:81/03:85/03:106-03:107/03:125/03:146/03:153/03:161/03:174/03:178/03:184-03:185/03:189-03:190/03:199/03:203/03:205/03:210-03:211/03:213N-03:215/03:220-03:221/03:224/03:229/03:233/03:240/03:245/03:247/03:251	DQ8(3), -, Null								8			11	
DQB1*03:02:03/03:02:23/03:37/03:175/03:223/03:237N, DQB1*06:37/06:139	DQ8(3), -, Null								8				
DQB1*03:02:04/03:02:13/03:204	DQ8(3), -								8			w	
DQB1*03:02:10/03:02:16/03:17:01/03:18/03:110/03:148-03:149/03:225/03:244	-											11	
DQB1*03:02:14	-								w			11	
DQB1*03:03:02:01-03:03:02:04/03:03:04-03:03:13/03:12/03:15/03:25-02-03:26/03:30-03:31/03:33-03:34/03:38-03:41/03:43/03:65/03:74/03:79/03:86-03:91Q/03:95N-03:99Q/03:104-03:105/03:111/03:113/03:117/03:123-03:124/03:126/03:141/03:145/03:155/03:168/03:176-03:177/03:200/03:209/03:212/03:222/03:227/03:230/03:234/03:239/03:248-03:249	DQ9(3), -, Null										10	11	
DQB1*03:03:03/03:06/03:20/03:25-01/03:112/03:136/03:156/03:238, DQB1*04:03:02, DQB1*06:09:04/06:15:01/06:22:01/06:22:03/06:51:01	DQ9(3), DQ3, -										10		
DQB1*03:04:01-03:04:03/03:14:01-03:14:02/03:80	DQ7(3), -						6	7				11	
DQB1*03:05:01-03:05:02/03:61/03:181/03:226/03:250	DQ8(3), -									9			
DQB1*03:05:03-03:05:04	DQ8(3)									9		11	
DQB1*03:08/03:228	-			3					8			11	
DQB1*03:36/03:122/03:151/03:171	-						w					11	
DQB1*03:70	-							7	8			11	
DQB1*03:78	-										w	11	

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12
DQB1*03:132	-									9			12
DQB1*03:137	-			3							10	11	
DQB1*03:179	-							w	8			11	
DQB1*03:194	-		2				6					11	
DQB1*04:01:01-04:02:01:03/04:02:03-04:02:07/04:02:09-04:02:12/04:04-04:09/04:11-04:30/04:32-04:39	DQ4, -, Null												12
DQB1*04:03:01	-										10		12
DQB1*04:10	-	w					6						12
DQB1*05:01:01:01-05:01:13/05:01:15/05:01:17-05:01:18/05:01:20-05:03:01:02/05:03:03-05:03:09/05:03:11-05:10/05:11:02-05:20/05:22-05:25/05:27-05:33/05:35-05:43:02/05:45-05:59/05:61-05:71/05:74-05:81/05:83-05:97/05:99-05:104/05:106-05:112/05:114-05:115/05:117-05:127/05:129/05:131-05:143	DQ5(1), -, Null	1											
DQB1*05:01:16/05:01:19/05:26/05:34/05:44/05:113/05:128N/05:130	-, Null	w											
DQB1*05:03:02	DQ5(1)	1					?						
DQB1*05:11:01	-	1										11	
DQB1*06:01:01-06:01:15/06:03:04/06:03:08-06:03:10/06:03:14/06:03:16/06:03:26/06:08:02-06:08:03/06:12/06:14:03/06:17/06:21/06:42-06:43/06:45/06:54N-06:57/06:59/06:64/06:91/06:98-06:105/06:108/06:120/06:132/06:140/06:145/06:157/06:177/06:181/06:190:01-06:190:02/06:194/06:196/06:205/06:209/06:214/06:229	DQ6(1), -, DQ1, Null		2										
DQB1*06:02:01:01-06:02:01:03/06:02:03-06:02:06/06:02:09-06:02:26/06:03:07/06:10-06:11:01/06:13:01-06:13:02/06:14:02/06:16/06:19:01-06:20/06:24/06:33/06:47/06:49-06:50/06:68/06:70-06:81/06:83-06:84/06:95/06:97/06:106-06:107/06:109/06:111-06:117/06:122/06:124-06:127/06:130-06:131/06:136-06:138/06:146:01-06:147/06:150-06:152/06:159/06:161/06:163/06:166/06:173-06:176/06:178-06:179N/06:182-06:183/06:188/06:192/06:197-06:198/06:200/06:206:01-06:206:02/06:208/06:211/06:213/06:215-06:216N/06:219/06:224-06:228	DQ6(1), -, DQ1, Null			3									
DQB1*06:02:02	DQ6(1)			3								11	
DQB1*06:02:07/06:03:01/06:03:03/06:03:06/06:03:11-06:03:13/06:03:15/06:03:17-06:03:18/06:03:20-06:03:25/06:08:01/06:11:02-06:11:03/06:14:01/06:26N/06:28/06:30-06:31/06:40-06:41/06:44/06:60-06:63/06:67/06:82/06:87/06:90/06:92/06:110/06:128/06:133-06:134/06:141/06:143-06:144N/06:148/06:154/06:165/06:170/06:184-06:185/06:187/06:191/06:195/06:199/06:203/06:210/06:218/06:221-06:223/06:230	-, DQ6(1), DQ1, Null		2	3									
DQB1*06:03:02	DQ6(1)		2	3								11	
DQB1*06:03:05/06:65	-		2	w									

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12
DQB1*06:04:01-06:04:07/06:04:09-06:07:02/06:09:01-01-06:09:03/06:09:05/06:18:01-06:18:02/ 06:22:02/06:25/06:27:01-06:27:02/06:32:01-06:32:02/06:34/06:36/06:38-06:39/06:52/06:58/06:66/ 06:69/06:85-06:86/06:88-06:89/06:93-06:94/06:118:01-06:118:02/06:129/06:135/06:155/06:158N/ 06:160/06:164/06:171-06:172/06:180/06:186/06:189/06:193N/06:202/06:204/06:207/06:217	DQ6(1), -, Null				4								
DQB1*06:04:08	-				4							11	
DQB1*06:09:06/06:121/06:212	-				w								
DQB1*06:15:02	-										w		
DQB1*06:23/06:156/06:162/06:169	-	1		3									
DQB1*06:29/06:123	-			3				8					
DQB1*06:35/06:53:01-06:53:02	-		2				6						
DQB1*06:46/06:96/06:119	-			3							10		
DQB1*06:142/06:168	-		2		4								
DQB1*06:149	-		w										
DQB1*06:201	-			w									

w = weak

? = nucleotide sequence information not available for the primer matching sequence

**Table 4:** Amplification patterns for all detectable **HLA-DQA1\*** specificities (**Lot-No DQA09-0**) based on IMGT/HLA Sequence Database Release 3.27.0, January 2017

Position						Mix	Allele	Serology	Size
H1	H3	H5	H7	H9	H11	Mix 1	DQA1*01:01:01:01-01:01:03/01:04:01:01-01:05:02/01:07Q/01:12	-	145 bp
G1	G3	G5	G7	G9	G11	Mix 2	DQA1*01:02:01:01-01:03:01:06/01:06/01:08-01:11/01:13-01:15N	-, Null	145 bp
F1	F3	F5	F7	F9	F11	Mix 3	DQA1*01:01:01:01-01:02:04/01:04:01:01-01:09/01:11-01:13	-	170 bp
E1	E3	E5	E7	E9	E11	Mix 4	DQA1*01:03:01:01-01:03:01:06/01:10/01:14-01:15N	-, Null	170 bp
D1	D3	D5	D7	D9	D11	Mix 5	DQA1*01:04:01:01-01:05:02/01:06?/01:07Q/01:08?-01:09?/01:12?-01:13?/01:15N?, DQA1*03:01:03?, DQA1*04:03N?-04:04?, DQA1*05:02?/05:04?/05:10?, DQA1*06:01:02?-06:02?	-, Null	200 bp
C1	C3	C5	C7	C9	C11	Mix 6	DQA1*02:01:01:01-02:01:01:02	-	105 bp
B1	B3	B5	B7	B9	B11	Mix 7	DQA1*03:01:01/03:01:03-03:03:02	-	130 bp
A1	A3	A5	A7	A9	A11	Mix 8	DQA1*04:01:01-04:04	-, Null	215 bp
H2	H4	H6	H8	H10	H12	Mix 9	<b>DQA1*05:01:01:01-05:09/05:10w/05:11</b>	-	<b>see below</b>
							DQA1*05:04	-	205 bp
							DQA1*05:01:01:01-05:09/05:10w/05:11	-	190 bp
G2	G4	G6	G8	G10	G12	Mix 10	DQA1*06:01:01-06:02	-	105 bp
F2	F4	F6	F8	F10	F12	Mix 11	Negative Control	-	none (440 bp)

w = weak

? = nucleotide sequence information not available for the primer matching sequence