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CTS Collaborative Transplant Study

WORKING INSTRUCTION
**HLA-DQB1*/DQA1* Low Resolution
CTS-PCR-SSP TRAY KITS**
LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following products:

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

**CHANGES COMPARED TO THE PREVIOUS LOT DQB09-0 FOR
HLA-DQB1* LOW RESOLUTION TYPING:**

- An update with the IMGT/HLA Sequence Database of April 2011 was performed; therefore, the mix specificities have been extended by new alleles. Please also note the new HLA nomenclature.

**CHANGES COMPARED TO THE PREVIOUS LOT DQA07-0 FOR
HLA-DQA1* LOW RESOLUTION TYPING:**

- An update with the IMGT/HLA Sequence Database of April 2011 was performed; therefore, the mix specificities have been extended by new alleles. Please also note the new HLA nomenclature.

Introduction

- Intended use: This kit reveals a low resolution typing of HLA-DQB1* and HLA-DQA1* by the PCR-SSP method.
- Allele coverage: IMGT/HLA Sequence Database Release 3.4.0, April 2011, except for HLA-DQB1*04:02:02 and DQB1*06:42 which are considered to be rare and can be detected by e. g. sequencing with the CTS-SEQUENCE KIT (you may contact us for further information).
- This manual is only valid for Lot No. **DQB09-1 DQA07-1**.
- This manual should be used together with the Main Manual (General Information) 'Working Instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No. 100A).

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1. Kit Composition

TRAY KIT

- Number of PCR primer mixes per test: 23 allele-specific mixes (13 mixes for HLA-DQB1* followed by 10 mixes for HLA-DQA1*) + one negative control mix.
- Number of tests per tray: 4
- Number of trays per kit: 10
- The primer mixes are aliquoted and lyophilized in thin-walled, yellow PCR-trays.
- PCR buffer: 3.0 ml of 5% Mastermix (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.

2. 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.

3. 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.

4. Result Evaluation

- 4.1 Check the approximate base pair size(s) of the PCR product(s) against the Primer Mix Specificity Table (Table 1 and 3) to confirm the correct product size(s).
- 4.2 Use the Typing Tables (Table 2 and 4) to make the allele assignments, or use the SCORE Software (www.IHWG.org) for detailed result interpretation.

5. 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 refer to Section 7 of the 'Working instruction for the CTS-PCR-SSP TRAY MINITRAY KITS' (Manual No.: 100A) supplied along with this product.

HLA-DQB1:

- Mix 6 and 7 of DQB1 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:

- The allele-specific band of **Mix 8**, if positive, may be less intense than the allele-specific bands produced by the other mixes.
- HLA-DQA1*0504 will show two PCR fragments with **Mix 9**.
- **Mix 10** (HLA-DQA1*060101-02) may show a slight cross-reaction with HLA-DQA1*0103.

6. 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.

7. 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.

8. 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:

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Hien Tran, M.D.

Table 1: Sizes of the PCR products and allele specificities of each HLA-DQB1* Low Resolution CTS-PCR-SSP primer mix (Lot No. DQB09-1) based on the IMGT/HLA Sequence Database Release 3.4.0, April 2011.

Amplification Control (Internal Positive Control): Product size 440 base pairs (bp)

Position on tray				Mix	Allele Specificity	Serology	Product sizes (bp)
H1	H4	H7	H10	Mix 1	DQB1*05:01:01:01-05:12, DQB1*06:23	DQ5, -, DQ6	see below
					DQB1*05:03:02, DQB1*06:23	DQ5, DQ6	135
					DQB1*05:01:01:01-05:12	DQ5, -	225
G1	G4	G7	G10	Mix 2	DQB1*06:01:01-06:01:06:06:03:01-06:03:02/06:08:01-06:08:02/06:11:02-06:12/06:14:01/06:17/06:21/06:26N/06:28/06:30-06:31/06:35/06:40-06:41/06:43-06:44	DQ6, -, DQ1, Null	see below
					DQB1*06:01:01-06:01:06:06:35/06:43	DQ6, -	160/170
					DQB1*06:03:01-06:03:02/06:08:01-06:08:02/06:11:02-06:12/06:14:01/06:17/06:21/06:26N/06:28/06:30-06:31/06:40-06:41/06:44	DQ6, DQ1, Null, -	170
F1	F4	F7	F10	Mix 3	DQB1*03:08, DQB1*06:02:01-06:03:02/06:08:01/06:10-06:11:02/06:13-06:14:02/06:16/06:19-06:20/06:23-06:24/06:26N/06:28-06:31/06:33/06:40-06:41/06:44	DQ8, DQ6, DQ1, Null, -	see below
					DQB1*03:08, DQB1*06:02:01-06:02:02/06:10-06:11:01/06:13/06:14:02/06:16/06:19-06:20/06:24/06:29/06:33	DQ8, DQ6, DQ1	165
					DQB1*06:02:01-06:03:02/06:08:01/06:10-06:11:02/06:13-06:14:02/06:16/06:20/06:23-06:24/06:26N/06:28-06:31/06:33/06:40-06:41/06:44	DQ6, DQ1, Null, -	105
E1	E4	E7	E10	Mix 4	DQB1*06:04:01-06:07:02/06:09/06:18/06:25/06:27/06:32/06:34/06:36/06:38-06:39	DQ6, -	170
D1	D4	D7	D10	Mix 5	DQB1*02:01:01-02:06	DQ2, -	200
C1	C4	C7	C10	Mix 6	DQB1*03:01:01:01-03:01:06/03:04/03:09/03:13-03:14/03:16/03:19/03:21-03:22/03:24/03:27-03:29/03:35, DQB1*06:35	DQ7, -, DQ3	105
B1	B4	B7	B10	Mix 7	DQB1*03:04/03:14	DQ7, DQ3	175
A1	A4	A7	A10	Mix 8	DQB1*03:02:01-03:02:05/03:07-03:08/03:11/03:32/03:37, DQB1*06:29/06:37	DQ8, -, DQ6	see below
					DQB1*03:02:01-03:02:05/03:07-03:08/03:11/03:32/03:37, DQB1*06:29	DQ8, -, DQ6	130
					DQB1*06:37	-	145
H2	H5	H8	H11	Mix 9	DQB1*03:05:01-03:05:04	DQ8	135
G2	G5	G8	G11	Mix 10	DQB1*03:03:02:01-03:03:03/03:06/03:12/03:15/03:20/03:25-03:26/03:30-03:31/03:33-03:34, DQB1*04:03:01-04:03:02, DQB1*06:15/06:22	DQ9, -, DQ3, DQ6	see below
					DQB1*03:03:02:01-03:03:03/03:06/03:12/03:15/03:20/03:25-03:26/03:30-03:31/03:33-03:34, DQB1*04:03:01-04:03:02,	DQ9, -, DQ3	130
					DQB1*06:15/06:22	DQ6	215
F2	F5	F8	F11	Mix 11	DQB1*03:23, DQB1*05:03:02?	-, DQ5	120
E2	E5	E8	E11	Mix 12	DQB1*03:01:01:01-03:01:01:03/03:01:03-03:01:05/03:02:01-03:02:02/03:02:04w/03:02:05-03:03:02/03:04/03:05-03:05:04/03:07-03:19/03:21-03:22/03:24/03:26/03:28-03:36, DQB1*06:02:02/06:03:02	DQ7, -, DQ8, DQ9, DQ3, DQ6	160
D2	D5	D8	D11	Mix 13	DQB1*04:01:01-04:02:01/04:03:01/04:04-04:08	DQ4, -	210

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 groups in the following rows depending on the fragment size)

Table 2: Amplification patterns of HLA-DQB1* alleles detected by the HLA-DQB1* Low Resolution CTS-PCR-SSP primer mixes (Lot No. DQB09-1) based on the IMGT/HLA Sequence Database Release 3.4.0, April 2011.

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12	13
DQB1*02:01:01-02:06	DQ2, -					5								
DQB1*03:01:01:01-03:01:01:03/03:01:03-03:01:05/03:09/03:13/03:16/03:19/03:21-03:22/03:24/03:28-03:29/03:35	DQ7, -						6						12	
DQB1*03:01:02/03:01:06/03:27	DQ7, -						6							
DQB1*03:02:01-03:02:02/03:02:05/03:07/03:11/03:32	DQ8, -								8				12	
DQB1*03:02:03/03:37, DQB1*06:37	DQ8, -								8					
DQB1*03:02:04	DQ8								8				w	
DQB1*03:03:02:01-03:03:02:03/03:12/03:15/03:26/03:30-03:31/03:33-03:34	DQ9, -										10		12	
DQB1*03:03:03/03:06/03:20/03:25, DQB1*04:03:02, DQB1*06:15/06:22	DQ9, DQ3, -, DQ6										10			
DQB1*03:04/03:14	DQ7, DQ3						6	7					12	
DQB1*03:05:01-03:05:02	DQ8									9				
DQB1*03:05:03-03:05:04	DQ8									9			12	
DQB1*03:08	DQ8			3					8				12	
DQB1*03:10/03:17-03:18/03:36	DQ3, DQ9, DQ8, -												12	
DQB1*03:23	-											11		
DQB1*04:01:01-04:02:01/04:04-04:08	DQ4, -													13
DQB1*04:03:01	-										10			13
DQB1*05:01:01:01-05:03:01:02/05:03:03-05:12	DQ5, -	1												
DQB1*05:03:02	DQ5	1										?		
DQB1*06:01:01-06:01:06/06:08:02/06:12/06:17/06:21/06:43	DQ6, -, DQ1		2											
DQB1*06:02:01/06:10-06:11:01/06:13/06:14:02/06:16/06:19-06:20/06:24/06:33	DQ6, DQ1			3										
DQB1*06:02:02	DQ6			3									12	
DQB1*06:03:01/06:08:01/06:11:02/06:14:01/06:26N/06:28/06:30-06:31/06:40-06:41/06:44	DQ6, DQ1, Null, -		2	3										
DQB1*06:03:02	DQ6		2	3									12	
DQB1*06:04:01-06:07:02/06:09/06:18/06:25/06:27/06:32/06:34/06:36/06:38-06:39	DQ6, -				4									
DQB1*06:23	DQ6	1		3										
DQB1*06:29	DQ6			3					8					
DQB1*06:35	-		2				6							

w = weak

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

Table 3: Sizes of the PCR products and allele specificities of each HLA-DQA1* Low Resolution CTS-PCR-SSP primer mix (Lot DQA07-1) based on the IMGT/HLA Sequence Database Release 3.4.0, April 2011.

Amplification Control (Internal Positive Control): Product size 440 base pairs

Position on tray				Mix	Allele Specificities	Product size (bp)
C2	C5	C8	C11	Mix 1	DQA1*01:01:01-01:01:02/01:04:01:01-01:05/01:07	145
B2	B5	B8	B11	Mix 2	DQA1*01:02:01:01-01:03:01:02/01:06	145
B2	B5	A8	A11	Mix 3	DQA1*01:01:01-01:02:04/01:04:01:01-01:07	170
H3	H6	H9	H12	Mix 4	DQA1*01:03:01:01-01:03:01:02	170
G3	G6	G9	G12	Mix 5	DQA1*01:04:01:01-01:05/01:06?-01:07?, DQA1*04:01:02?-04:04?, DQA1*05:02?/05:04?/05:10?, DQA1*06:01:02?-06:02?	200
F3	F6	F9	F12	Mix 6	DQA1*02:01	105
E3	E6	E9	E12	Mix 7	DQA1*03:01:01-03:03	110
D3	D6	D9	D12	Mix 8	DQA1*04:01:01-04:04	170
C3	C6	C9	C12	Mix 9	DQA1*05:01:01:01-05:09/05:11	see below
					DQA1*05:04	205
					DQA1*05:01:01:01-05:09/05:11	190
B3	B6	B9	B12	Mix 10	DQA1*06:01:01-06:02	170
A3	A6	A9	A12	Mix 11	Negative Control	none (440bp)

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

(results need to be confirmed by HLA-DQA1* High Resolution Typing)

Table 4: Amplification patterns for all HLA-DQA1* alleles detected by the HLA-DQA Low Resolution CTS PCR-SSP primer mixes (Lot DQA07-1) based on the IMGT/HLA Sequence Database Release 3.4.0, April 2011.

Amplification Control (Internal Positive Control): Product size 440 base pairs

Allele	1	2	3	4	5	6	7	8	9	10
DQA1*01:01:01:01:01:02	1		3							
DQA1*01:02:01:01:01:02:04		2	3							
DQA1*01:03:01:01:01:03:01:02		2		4						
DQA1*01:04:01:01:01:05	1		3		5					
DQA1*01:06		2	3		?					
DQA1*01:07	1		3		?					
DQA1*02:01						6				
DQA1*03:01:01:03:03							7			
DQA1*04:01:01								8		
DQA1*04:01:02-04:04					?			8		
DQA1*05:01:01:01-05:01:01:02/05:03/05:05:01:01-05:09/05:11									9	
DQA1*05:02/05:04					?				9	
DQA1*05:10					?					
DQA1*06:01:01										10
DQA1*06:01:02-06:02					?					10

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

(results need to be confirmed by HLA-DQA1* High Resolution Typing)