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Manual No.: 19
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Lot No.: DQB09-1

CTS *Collaborative Transplant Study*

**WORKING INSTRUCTION
 HLA-DQB1* Low Resolution
 CTS-PCR-SSP TRAY KIT**

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following products:

Product No.	Description
119	HLA-DQB1 low resolution CTS-PCR-SSP TRAY KIT  

CHANGES COMPARED TO LOT DQB09-0:

- 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 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**.
- 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: 14 (13 allele-specific mixes and 1 negative control mix)
- Number of tests per tray: 6
- Number of trays per kit: 10
- The primer mixes are aliquoted and lyophilized in thin-walled, green PCR-Trays.
- PCR buffer: 3 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 size of the PCR product against the Primer Mix Specificity Table (Table 1) to confirm the correct product size.
- 4.2 Use the Amplification Pattern Table (Table 2) to make allele assignment. Alternatively, you can 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 and MINITRAY KITS' (Manual No. 100A) supplied along with this product.

SPECIAL NOTES

❖ 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.

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 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 size (bp)
H1	H3	H5	H7	H9	H11	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	G3	G5	G7	G9	G11	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	F3	F5	F7	F9	F11	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	E3	E5	E7	E9	E11	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	D3	D5	D7	D9	D11	Mix 5	DQB1*02:01:01-02:06	DQ2, -	200
C1	C3	C5	C7	C9	C11	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	B3	B5	B7	B9	B11	Mix 7	DQB1*03:04/03:14	DQ7, DQ3	175
A1	A3	A5	A7	A9	A11	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	H4	H6	H8	H10	H12	Mix 9	DQB1*03:05:01-03:05:04	DQ8	135
G2	G4	G6	G8	G10	G12	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	F4	F6	F8	F10	F12	Mix 11	DQB1*03:23, DQB1*05:03:02?	-, DQ5	120
E2	E4	E6	E8	E10	E12	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/03:02:05-03:03:02:03/03:04/03:05:03-03:05:04/03:07-03:19/03:21-03:22/03:24/03:26/03:28-03:36, DQB1*06:02:02/06:03:02	DQ7, -, DQ8, DQ9, DQ3, DQ6	160
D2	D4	D6	D8	D10	D12	Mix 13	DQB1*04:01:01-04:02:01/04:03:01/04:04-04:08	DQ4, -	210
C2	C4	C6	C8	C10	C12	Mix 14	Negative control	-	none (440)

Positions B2-A2, B4-A4, B6-A6, B8-A8, B10-A10 and B12-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 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 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