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CTS Collaborative Transplant Study



WORKING INSTRUCTION

HLA-DQA1* Low Resolution CTS-PCR-SSP TRAY KITS

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following products:

Product No.	Description
127	HLA-DQA1* Low Resolution CTS-PCR-SSP Tray Kit

MAIN CHANGES COMPARED TO THE LOT DQA07-0:

- 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-DQA1* by the PCR-SSP method.
- Allele coverage: IMGT/HLA Sequence Database Release 3.4.0, April 2011.
- This manual is only valid for Lot No. DQA07-1.
- 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).

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1. Kit Composition

TRAY KIT

- Number of PCR primer mixes per test: 11 (10 allele-specific and 1 negative control mix)
- Number of tests per tray: 6
Please note: Well positions E2 - A2, E4 - A4, E6 - A6, E8 - A8, E10 - A10, E12 - A12 are empty!
- Number of trays per kit: 10
- The primer mixes are aliquoted and lyophilized in thin-walled, blue PCR-trays.
- PCR buffer: 3.0 ml of 5.0% 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 of the PCR product against the Primer Mix Specificity Table (Table 1) to confirm the correct product size.
- 4.2 Use the Reaction Pattern Tables (Table 2) to make the allele assignments. 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

- 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 weak 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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Table 1: Fragment sizes and allele specificities for each HLA-DQA1* low resolution 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)
H1	H3	H5	H7	H9	H11	Mix1	DQA1*01:01:01-01:01:02/01:04:01:01-01:05/01:07	145
G1	G3	G5	G7	G9	G11	Mix2	DQA1*01:02:01:01-01:03:01:02/01:06	145
F1	F3	F5	F7	F9	F11	Mix3	DQA1*01:01:01-01:02:04/01:04:01:01-01:07	170
E1	E3	E5	E7	E9	E11	Mix4	DQA1*01:03:01:01-01:03:01:02	170
D1	D3	D5	D7	D9	D11	Mix5	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
C1	C3	C5	C7	C9	C11	Mix6	DQA1*02:01	105
B1	B3	B5	B7	B9	B11	Mix7	DQA1*03:01:01-03:03	110
A1	A3	A5	A7	A9	A11	Mix8	DQA1*04:01:01-04:04	170
H2	H4	H6	H8	H10	H12	Mix9	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
G2	G4	G6	G8	G10	G12	Mix10	DQA1*06:01:01-06:02	170
F2	F4	F6	F8	F10	F12	Mix 11	Negative Control	none (440bp)

Well positions E2 - A2, E4 - A4, E6 - A6, E8 - A8, E10 - A10, E12 - A12 are empty!

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

Table 2: Expected reactions for the most common HLA-DQA1* alleles with the CTS PCR-SSP reagents (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