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Manual No.	27
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Product No.	127
Lot No.	DQA10-3


CTS Collaborative Transplant Study

WORKING INSTRUCTION

HLA-DQA1* CTS-PCR-SSP TRAY KIT

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following product:

Product No.	Description
127	HLA-DQA1* low resolution CTS-PCR-SSP Tray Kit 

1. Main differences

- **Between Lot DQA10-3 (the current lot) and Lot DQA10-2:**
The kit was updated to cover new alleles included in the IMGT/HLA Database of January 2023. Deleted and renamed alleles were taken into consideration.

2. 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 3.51.0, January 2023, except: DQA1* 01:20, 03:29
- This manual is only valid for **Lot No. DQA10-3**.
- 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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4. Kit Composition

- Number of PCR primer mixes per test: 11:
 - 10 HLA-specific mixes
 - 1 negative control mix
- Please note: Well positions E2 - A2, E4 - A4, E6 - A6, E8 - A8, E10 - A10, E12 - A12 are empty!
- Number of tests per tray: 6
- Number of trays per kit: 10
- The primer mixes are aliquoted and dried in 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 Table (Appendix / Table 1) to confirm the correct product size.
- Use the Amplification Pattern Table (Appendix / Table 2) to make the allele assignments or use the SCORE Software for detailed result interpretation.

8. Interpretation Hints

- Weak or false positive reactions can occur if you use a different Taq polymerase. Also the quality and quantity of DNA is a crucial factor and can affect the mix reactivities. Under suboptimal test conditions, some mixes could give rise to false positive reactions (if there are any potentially false positive reactions when using this specific lot, they are indicated in Section 9).
- Alleles that are known to amplify weakly are listed with "w" (= weak) in the tables (Appendix).
- Please also refer to Section 7 and 8 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-DQA1*05:04 generates two PCR fragments with Mix 9.

Potentially false positive reactions: Mix 5

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 us at:

Phone: +49 6221 564013; Fax: +49 6221 564200

E-mail: dna.labor@med.uni-heidelberg.de

13. Appendix

Table 1: Allele specificities and sizes of the PCR products of each **HLA-DQA1*** CTS-PCR-SSP primer mix (**Lot No. DQA10-3**) based on IMGT/HLA Sequence Database Release 3.51.0, January 2023

Position						Mix	Allele	Serology	Size
H1	H3	H5	H7	H9	H11	Mix 1	DQA1*01:01:01:01-01:01:01:03/01:01:01:05-01:01:11/01:04:01:01-01:05:04/01:07Q/01:12/01:18/01:22/01:26-01:27/01:29w/01:34-01:35/01:37/01:43/01:49/01:53/01:55-01:56/01:59-01:61/01:64/01:66-01:67/01:74/01:77/01:80/01:83/01:86/01:88N-01:90/01:95-01:96/01:98-01:99/01:107/01:110	-, Null	145 bp
G1	G3	G5	G7	G9	G11	Mix 2	DQA1*01:02:01:01-01:03:08/01:06/01:08-01:11/01:13-01:17/01:19/01:21/01:23-01:25/01:28/01:30-01:33/01:36/01:38:01:01-01:42/01:44-01:48/01:50-01:52/01:54/01:57-01:58/01:62-01:63:01:02/01:65/01:68-01:73/01:75-01:76/01:78-01:79/01:81-01:82/01:84-01:85/01:87:01:01-01:87:01:02/01:91/01:92?/01:93-01:94/01:97/01:100-01:106/01:108-01:109/01:111	-, Null	145 bp
F1	F3	F5	F7	F9	F11	Mix 3	DQA1*01:01:01:01-01:01:01:03/01:01:01:05-01:02:18/01:04:01:01-01:04:05/01:04:06?-01:04:07?/01:04:08-01:09/01:11-01:13/01:16N/01:18-01:19/01:21-01:23/01:25-01:28/01:31-01:32/01:34-01:43/01:46/01:48-01:49/01:51:01:01-01:56/01:58-01:59/01:60?/01:61-01:64/01:66-01:67/01:69/01:71-01:74/01:75?/01:77/01:80-01:81/01:83/01:85-01:86/01:88N-01:91/01:92?/01:93-01:96/01:98-01:100/01:103-01:107/01:109-01:111	-, Null	170 bp
E1	E3	E5	E7	E9	E11	Mix 4	DQA1*01:03:01:01-01:03:08/01:10/01:14-01:15N/01:17/01:24/01:30/01:33/01:44-01:45/01:47/01:50/01:57/01:65/01:68/01:70/01:76/01:78-01:79/01:82/01:84?/01:87:01:01-01:87:01:02/01:97/01:102/01:108	-, Null	170 bp
D1	D3	D5	D7	D9	D11	Mix 5	DQA1*01:03:02?-01:03:03?/01:03:05?/01:04:01:01-01:04:02:02/01:04:03?-01:04:04?/01:04:05-01:05:04/01:06?/01:07Q/01:08?-01:09?/01:12?-01:13?/01:15N?/01:17?-01:18?/01:20?/01:22?/01:26?/01:28?-01:29?/01:31?-01:37?/01:40Q?/01:42?/01:46?-01:47?/01:50?/01:52?-01:53?/01:55?-01:56?/01:59?-01:62?/01:64/01:67?-01:75?/01:77?-01:79?/01:80/01:81?/01:83?/01:85?/01:86/01:90?-01:98?/01:100/01:101?/01:103?/01:108?-01:111?, DQA1*02:01:02?/02:18?-02:24?, DQA1*03:01:03?/03:19?/03:22?-03:27N?/03:29?-03:30?, DQA1*04:01:04?-04:01:05?/04:03N?-04:04?/04:09?-04:12N?, DQA1*05:02?/05:04?/05:10?/05:32?/05:34?-05:35:01?/05:37?/05:39?-05:46?/05:50?/05:52?, DQA1*06:01:02?-06:04?	-, Null	200 bp
C1	C3	C5	C7	C9	C11	Mix 6	DQA1*02:01:01:01-02:05/02:06/02:07-02:31	-, Null	105 bp
B1	B3	B5	B7	B9	B11	Mix 7	DQA1*03:01:01:01/03:01:01:03/03:01:03-03:28/03:29?/03:30-03:44	-, Null	130 bp
A1	A3	A5	A7	A9	A11	Mix 8	DQA1*01:02:12, DQA1*04:01:01:01-04:17	-, Null	215 bp

Position						Mix	Allele	Serology	Size
H2	H4	H6	H8	H10	H12	Mix 9	DQA1*05:01:01:01-05:01:02/05:01:04-05:09:01:02/05:10w/05:11:01:01-05:33/05:34?/05:35:01-05:48/05:49?/05:50-05:74	-, Null	see below
							DQA1*05:04	-	205 bp
							DQA1*05:01:01:01-05:01:02/05:01:04-05:09:01:02/05:10w/05:11:01:01-05:33/05:34?/05:35:01-05:48/05:49?/05:50-05:74	-, Null	190 bp
G2	G4	G6	G8	G10	G12	Mix 10	DQA1*06:01:01:01-06:04	-	105 bp
F2	F4	F6	F8	F10	F12	Mix 11	-	-	None (440bp)

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

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

w = weak

? = nucleotide sequence information not available for the primer matching sequence or alleles with unknown reactivities

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 2: Amplification patterns of HLA-DQA1* alleles detected by the HLA-DQA1* CTS-PCR-SSP primer mixes (Lot No. DQA10-3) based on IMGT/HLA Sequence Database Release 3.51.0, January 2023

Allele	Serology	1	2	3	4	5	6	7	8	9	10
DQA1*01:01:01:01-01:01:01:03/01:01:01:05-01:01:11/01:27/01:43/01:49/01:66/01:88N-01:89/01:99/01:107	-, Null	1		3							
DQA1*01:02:01:01-01:02:11/01:02:13-01:01:02:18/01:11/01:16N/01:19/01:21/01:23/01:25/01:38:01:01-01:39/01:41/01:48/01:51:01:01-01:51:01:02/01:54/01:58/01:63:01:01-01:63:01:02/01:104-01:106	-, Null		2	3							
DQA1*01:02:12	-		2	3					8		
DQA1*01:03:01:01-01:03:01:13/01:03:04/01:03:06-01:03:08/01:10/01:14/01:24/01:30/01:44-01:45/01:57/01:65/01:76/01:82/01:87:01:01-01:87:01:02/01:102	-		2		4						
DQA1*01:03:02-01:03:03/01:03:05/01:15N/01:17/01:33/01:47/01:50/01:68/01:70/01:78-01:79/01:97/01:108	-, Null		2		4	?					
DQA1*01:04:01:01-01:04:02:02/01:04:05/01:04:08-01:05:04/01:07Q/01:64/01:80/01:86	-	1		3		5					
DQA1*01:04:03-01:04:04/01:12/01:18/01:22/01:26/01:34-01:35/01:37/01:53/01:55-01:56/01:59/01:61/01:67/01:74/01:77/01:83/01:90/01:95-01:96/01:98/01:110	-	1		3		?					
DQA1*01:04:06-01:04:07	-	1		?		5					
DQA1*01:06/01:08-01:09/01:13/01:28/01:31-01:32/01:36/01:40Q/01:42/01:46/01:52/01:62/01:69/01:71-01:73/01:81/01:85/01:91/01:93-01:94/01:103/01:109/01:111	-		2	3		?					
DQA1*01:20	-					?					
DQA1*01:29	-	w				?					
DQA1*01:60	-	1		?		?					
DQA1*01:75	-		2	?		?					
DQA1*01:84	-		2		?						
DQA1*01:92	-		?	?		?					
DQA1*01:100	-		2	3		5					
DQA1*01:101	-		2			?					
DQA1*02:01:01:01-02:01:01:05/02:01:03-02:05/02:07-02:17/02:25-02:31	-, Null						6				
DQA1*02:01:02/02:18-02:24	-					?	6				
DQA1*03:01:01:01/03:01:01:03/03:01:04-03:18/03:20-03:21/03:28/03:31-03:44	-, Null							7			
DQA1*03:01:03/03:19/03:22-03:27N/03:30	-, Null					?		7			
DQA1*03:29	-					?		?			
DQA1*04:01:01:01-04:01:03/04:01:06-04:02/04:05-04:08/04:13-04:17	-, Null								8		
DQA1*04:01:04-04:01:05/04:03N-04:04/04:09-04:12N	-, Null					?			8		

Allele	Serology	1	2	3	4	5	6	7	8	9	10
DQA1*05:01:01:01-05:01:02/05:01:04-05:01:11/05:03:01:01-05:03:02/05:05:01:01-05:09:01:02/05:11:01:01-05:31/05:33/05:35:02-05:36N/05:38/05:47-05:48/05:51/05:53-05:74	-, Null									9	
DQA1*05:02/05:04/05:32/05:35:01/05:37/05:39-05:46/05:50/05:52	-					?				9	
DQA1*05:10	-					?				w	
DQA1*05:34	-					?				?	
DQA1*05:49	-									?	
DQA1*06:01:01:01-06:01:01:04	-										10
DQA1*06:01:02-06:04	-					?					10

w = weak

? = nucleotide sequence information not available for the primer matching sequence or alleles with unknown reactivities

14. Certificate of Analysis



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Product number	127
Lot number	DQA10-3
Number of HLA-specific primer mixes per test	10

Mix specifications

The specificity of each primer pair has been tested against a panel of well characterized DNAs.

Result

No false positive or false negative amplifications were obtained under our test conditions of the bulk reagents.

Date of approval	09.03.2023
Approved by	H. Tran, M.D. Quality Control, Supervisor