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Manual No.: 30 Revision: February 1, 2008 Product No.: 130 Lot No.: DQBH03-1

CTS *Collaborative Transplant Study*

WORKING INSTRUCTION

HLA-DQB1* High Resolution - Part I **(HLA-DQB1*02 - *05 High Resolution Typing)** **CTS-PCR-SSP TRAY KIT**

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following products:

Product No.	Description
130	HLA-DQB1* High Resolution (Part I) CTS-PCR-SSP Tray Kit <div style="float: right; text-align: right;"> IVD CE </div>

CHANGES COMPARED TO LOT DQBH02:

This kit has thoroughly been revised to cover new alleles. For this purpose, the composition of several mixes of the previous lot has been modified. The total number of mixes (24) remains unchanged.

- Mix No. 4 also detects HLA-DQB1*0311.
- Mix No. 5, 7 and 21: completely different specificities than previously!
- Mix No. 6 detects additional HLA-DQB1*06 alleles (but still DQB1*06 group only).
- Mix No. 8 also detects HLA-DQB1*0202 and *0204.
- Mix No. 12 also detects HLA-DQB1*0316.
- Mix No. 17 also detects HLA-DQB1*0306.
- Mix No. 19 gives weakly positive signals with HLA-DQB1*0401, *0402 and *0619.

Introduction

- Intended use: This kit reveals a high resolution typing of HLA-DQB1*02 - DQB1*05 by the PCR-SSP method. **For subtyping of HLA-DQB1*06 alleles, please use the HLA-DQB1 (**Part II**) CTS-PCR-SSP Tray Kit. Alternatively, use HLA-DQB1* High Resolution CTS-PCR-SSP Tray Kit (Product No. 105) which contains primer mixes for high resolution typing of all HLA-DQB1 alleles.**
- Allele coverage: IMGT/HLA Sequence Database Release 2.11, October 2005.
- This manual is only valid for Lot No. **DQBH03-1**.
- This manual should be used together with the Main Manual (General Information) the 'Working Instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No. 100A).

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 (HLA-DQB1*02 - *05 Subtyping) / Lot No.: DQBH03-1

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1. Kit Composition

TRAY KIT

- Number of PCR primer mixes per test: 24 (24 allele-specific mixes)
- Number of tests per tray: 4
- Number of trays per kit: 10
- The primer mixes are aliquoted and lyophilized in thin-walled, brown PCR-trays.
- PCR buffer: 3 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(s) of the PCR product(s) against the Primer Mix Specificity Table (Table 1) to confirm the correct product size(s).
- 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.

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 current lot DQBH03-1 may require less Taq polymerase than the previous lots. Depending on the brand and lot of Taq polymerase, a reduction of roughly 10 to 20% of the normally used Taq amount may be tried in the validation procedure.
- HLA-DQB1*0601 may occasionally cause weak false-positive reaction with mix 12.

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 and 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: Fragment sizes and allele specificities for each HLA-DQB1* High Resolution (**Part I**) PCR-SSP primer mix (**Lot DQBH03-1**) based on IMGT/HL Sequence Database Release 2.11, October 2005. **Amplification Control (Internal Positive Control):** Product size 440 base pairs

Position on tray				Mix	Allele Specificities	Product size (base pairs)
H1	H4	H7	H10	Mix 1	DQB1*050101-0301/0302/04	225
G1	G4	G7	G10	Mix 2	DQB1*050101/0102	135
F1	F4	F7	F10	Mix 3	DQB1*050201/0202/04	135
E1	E4	E7	E10	Mix 4	DQB1*0311, DQB1*0504:	see below
					DQB1*0504	200
					DQB1*0311	250
D1	D4	D7	D10	Mix 5	DQB1*0313	165
C1	C4	C7	C10	Mix 6	DQB1*060101/0102/0103-0501/0502/06/07/08/09-12/13/14-19/20/21-27:	see below
					DQB1*060401-0501/0502/06/07/09/12/15/17/21/22/25	260
					DQB1*0602/03/06/08/10-1102/13/14/16/18/19/20/23/24/26N/27	260
					DQB1*060101/0102/0103	225
B1	B4	B7	B10	Mix 7	DQB1*030101/0102/04/09/13/15/16:	see below
					DQB1*030101/0102/04/09/13/16	135
					DQB1*0315	155
A1	A4	A7	A10	Mix 8	DQB1*020101/0102w/02/04	120
H2	H5	H8	H11	Mix 9	DQB1*0202/03, DQB1*060501?-08?/10?-1102?/13?-27?	85
G2	G5	G8	G11	Mix 10	DQB1*030101/0102/04/09/10/13/14/16	215
F2	F5	F8	F11	Mix 11	DQB1*030101/0102/09/10/13/16, DQB1*060101-0103	135
E2	E5	E8	E11	Mix 12	DQB1*0304/14/16:	see below
					DQB1*0316	130
					DQB1*0304/14	135
D2	D5	D8	D11	Mix 13	DQB1*0309	170
C2	C5	C8	C11	Mix 14	DQB1*0310, DQB1*060101/0102w/0103	105
B2	B5	B8	B11	Mix 15	DQB1*030201/0202/0503/07/08/11	130
A2	A5	A8	A11	Mix 16	DQB1*020101-04, DQB1*0307:	see below
					DQB1*020101-04	200
					DQB1*0307	145
H3	H6	H9	H12	Mix 17	DQB1*0306/08, DQB1*0602/10/1101/13/16/19/20:	see below
					DQB1*0306	155
					DQB1*0308, DQB1*0602/10/1101/13/16/19/20	165
G3	G6	G9	G13	Mix 18	DQB1*0203, DQB1*030302/0303/06/12/15	135
F3	F6	F9	F12	Mix 19	DQB1*030201-0303/0501-06/07w/08/11/15/17, DQB1*0401w/02w DQB1*050202/0301/0302, DQB1*0619w	145
E3	E6	E9	E12	Mix 20	DQB1*030501/0503	150
D3	D6	D9	D12	Mix 21	DQB1*0204, DQB1*060501?-08?/10?-1102?/13?-27?	140
C3	C6	C9	C12	Mix 22	DQB1*030101/0201-0302/04/0503/07-17	160
B3	B6	B9	B12	Mix 23	DQB1*0401	205
A3	A6	A9	A12	Mix 24	DQB1*0402	205

w = weak

? = nucleotide sequence information not available for primer matching

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 for all HLA-DQB1* alleles detected by the HLA-DQB1 High Resolution (**Part I**) CTS PCR-SSP primer mixes (**Lot DQBH03-1**) based on IMGT/HLA Sequence Database Release 2.11, October 2005

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

Allele	Mix	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
DQB1*020101/0102									+								+								
DQB1*020102									W								+								
DQB1*0202									+								+								
DQB1*0203										+							+		+						
DQB1*0204									+								+					+			
DQB1*030101								+			+	+											+		
DQB1*030102								+			+	+												+	
DQB1*030201/0202																+				+	+			+	
DQB1*030302																			+	+				+	
DQB1*030303																			+	+					
DQB1*0304								+			+		+											+	
DQB1*030501																				+	+				
DQB1*030502																				+					
DQB1*030503																+				+	+			+	
DQB1*0306																+			+	+		+		+	
DQB1*0307																+	+			W				+	
DQB1*0308																+		+		+				+	
DQB1*0309								+			+	+		+										+	
DQB1*0310											+	+												+	
DQB1*0311				+							+	+			+					+				+	
DQB1*0312																+			+					+	
DQB1*0313					+			+			+	+												+	
DQB1*0314											+		+											+	
DQB1*0315								+											+	+				+	
DQB1*0316								+			+	+	+						+	+				+	
DQB1*0317																				+				+	
DQB1*0401																				W					+
DQB1*0402																				W					+
DQB1*050101/0102		+	+																						
DQB1*050201		+		+																					
DQB1*050202		+		+																	+				
DQB1*050301/0302		+																			+				
DQB1*0504		+		+	+																+				
DQB1*060101-0103								+				+			+										
DQB1*060102								+			+				W										
DQB1*0602								+											+						
DQB1*0603-0402/09/12								+																	
DQB1*060501-08/1102/14/15/17/18/21-27								+															?		
DQB1*0610/1101/13/16/20								+				?							+				?		
DQB1*0619								+				?							+		W		?		
	Mix	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

w = weak

? = nucleotide sequence information not available for primer matching