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Manual No.: 11
Revision: May 09, 2011
Product No.: 211
Lot No.: DR0812L03-1

CTS Collaborative Transplant Study

WORKING INSTRUCTION
HLA-DRB1*08/12 SUBTYPING
CTS-PCR-SSP MINITRAY KITS

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following products:

Product No.	Description
211	HLA-DRB1*08/12 Subtyping CTS-PCR-SSP Minitray Kit  

Main differences between Lot DR0812L03-1 (the current lot) and Lot DR0812L03:

- 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.

Main differences between Lot DR0812L03-0 and Lot DR0812L02

- The total number of primer mixes has been increased from 16 to **24 (23 allele-specific mixes and one negative control mix)**.
- Mixes **17 to 23** are new allele-specific mixes.

Introduction

- Intended use: This kit reveals a high resolution typing of HLA-DRB1*08 and HLA-DRB1*12 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. DR0812L03-1.
- This manual should be used together with the Main Manual (General Information Sheet) the 'Working Instruction for the CTS-PCR-SSP **TRAY and MINITRAY KITS**' (Manual No. 100A).

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1. Kit Composition

MINITRAY KIT

- Number of PCR primer mixes per test (minitray): 24 (23 allele-specific mixes and 1 negative control mix)
- Number of tests (minitrays) per kit: 12
- The primer mixes are aliquoted and lyophilized in thin-walled, purple PCR-minitrays.
- PCR buffer: 1.0 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 size of the PCR product against the Primer Mix Specificity Table (Table 1) to confirm the correct product size.
- 4.2 Use the Amplification Table (Table 2) to make allele assignment 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 and MINITRAY KITS' (Manual No. 100A) supplied along with this product.

Special notes

- Some common HLA-DRB1*11, -DRB1*13, -DRB1*14 and -DRB1*16 specificities share relevant specific sequence motives with HLA-DRB1*08 and -DRB1*12 alleles and therefore will react positively with few mixes (see Table 2). Please take this into consideration while you are interpreting your reaction patterns!

6. Trouble Shooting

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-DRB1*08/12 SUBTYPING CTS-PCR-SSP primer mix (Lot No. DR0812L03-1) based on the IMGT/HLA Sequence Database Release 3.4.0, April 2011

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

Mix	Amplified Alleles	Serology	Product Size
Mix 1	*08:09/08:21/08:32/08:35, 11:13:01-11:13:02/11:17/11:52, 12:01:01/12:02:01/12:02:04-12:02:05/12:04-12:13/12:17-12:21/12:23-12:25/12:27, 13:76, 14:01:01/14:04-14:05:02/14:07:01-14:08/14:10-14:11/14:14-14:15/14:23:01-14:23:03/14:26/14:28/14:31/14:32:02/14:34-14:36/14:39-14:40/14:43-14:44:01/14:45/14:54-14:56/14:60-14:62/14:65/14:68/14:71-14:73/14:77/14:82/14:84/14:86-14:88/14:90/14:96/14:99-14:101/14:104-14:105/14:107/14:110-14:113, DRB8*01:01?	DR8, Undefined, -, DR11, DR14, DR12, Null, DR13, DR1404/DR14, DR6	see below
	*08:09/08:21/08:32/08:35, 14:15/14:40/14:77/14:84, DRB8*01:01?	DR8, -, DR14	150 bp
	*11:13:01-11:13:02/11:17/11:52, 12:01:01/12:02:01/12:02:04-12:02:05/12:04-12:13/12:17-12:21/12:23-12:25/12:27, 13:76, 14:01:01/14:04-14:05:02/14:07:01-14:08/14:10-14:11/14:14/14:23:01-14:23:03/14:26/14:28/14:31/14:32:02/14:34-14:36/14:39/14:43-14:44:01/14:45/14:54-14:56/14:60-14:62/14:65/14:68/14:71-14:73/14:82/14:86-14:88/14:90/14:96/14:99-14:101/14:104-14:105/14:107/14:110-14:113, DRB8*01:01?	DR11, DR14, DR12, DR13, DR1404/DR14, DR6, -, Null	165 bp
Mix 2	*08:07/08:12/08:22w, 12:01:01-12:02:05/12:04-12:15/12:17-12:18/12:21/12:23-12:27, 14:28	DR8, DR12, -, Null, DR14	see below
	*08:07	DR8	165 bp
	*08:12/08:22w, 12:01:01-12:02:05/12:04-12:15/12:17-12:18/12:21/12:23-12:27, 14:28	DR8, DR12, -, Null, DR14	250 bp
Mix 3	*12:01:01/12:01:03-12:02:03/12:02:05-12:04/12:05w/12:06-12:13/12:17-12:21/12:23-12:25/12:27	DR12, -, Null	145 bp
Mix 4	*08:03:02/08:10/08:12/08:14-08:15/08:18-08:19/08:23/08:25/08:27/08:29-08:30:02/08:32-08:38/08:45, 12:01:01-12:01:02/12:03:02-12:11, 13:17	DR8, Undefined, -, DR12, DR13	195 bp
Mix 5	*08:01:01-08:02:03/08:04:01-08:09/08:11/08:16-08:17/08:21-08:22/08:24/08:26/08:28/08:31/08:39/08:41-08:44, 11:67, 12:02:01-12:02:02/12:02:04/12:11/12:13/12:16, 14:15/14:73	DR8, -, DR8/DR11, DR12	see below
	*12:11	DR12	135 bp
	*08:01:01-08:02:03/08:04:01-08:09/08:11/08:16-08:17/08:21-08:22/08:24/08:26/08:28/08:31/08:39/08:41-08:44, 11:67, 12:02:01-12:02:02/12:02:04/12:13/12:16, 14:15/14:73	DR8, -, DR8/DR11, DR12	195 bp

Mix	Amplified Alleles	Serology	Product Size
Mix 6	*08:02:01-08:02:03/08:04:01-08:04:05/08:04:07/08:09/08:13/08:15/08:21/08:24/08:28/08:30:01/08:31/08:41-08:42/08:44-08:45, 11:67, 12:04/12:09, 13:17/13:116, 14:11/14:15/14:52	DR8, -, DR8/DR11, DR12, DR13, DR14	see below
	*08:02:01-08:02:03/08:04:01-08:04:05/08:04:07/08:09/08:13/08:15/08:21/08:24/08:28/08:30:01/08:42/08:44-08:45, 12:09, 13:17/13:116, 14:15/14:52	DR8, -, DR12, DR13, DR14	165 bp
	*08:31/08:41, 11:67, 12:04, 14:11	DR8/DR11, -, DR12, DR14	170 bp
Mix 7	*08:09w/08:21w, 12:05/12:15, 14:04w/14:11w/14:15w/14:28w/14:31w/14:50w	DR8, DR12, DR1404/DR14	105 bp
Mix 8	*08:05/08:18/08:24-08:25/08:31/08:40-08:41, 12:01:01-12:02:02/12:03:02-12:13/12:16, 13:17/13:116, 14:31/14:52	DR8, DR8/DR11, -, DR12, DR13, DR14	215 bp
Mix 9	*08:01:01-08:01:05/08:03:02/08:05-08:06/08:10/08:12/08:14/08:16-08:18/08:22-08:23/08:26-08:27/08:29/08:32-08:33/08:35-08:40/08:43	DR8, Undefined, -	165 bp
Mix 10	*08:01:01-08:04:07/08:06-08:17/08:19-08:23/08:26-08:28/08:30:01-08:30:02/08:32-08:39/08:42/08:44-08:45, 11:23/11:25/11:45/11:55/11:64/11:67, 13:13/13:18/13:47/13:55, 14:03:01-14:03:02/14:12:01-14:12:02/14:15/14:27/14:40/14:55w/14:63/14:67/14:77-14:78/14:84-14:85/14:89/14:102	DR8, -, undefined, DR11, DR13, DR1403/DR14, DR6	see below
	*08:04:02/08:20, 11:23/11:25/11:45/11:55/11:64, 13:13/13:18/13:47/13:55, 14:03:01-14:03:02/14:12:01-14:12:02/14:27/14:40/14:55w/14:63/14:67/14:77-14:78/14:84-14:85/14:89/14:102	DR8, undefined, DR11, -, DR13, DR1403/DR14, DR6	225 bp
	*08:01:01-08:04:07/08:06-08:17/08:19/08:21-08:23/08:26-08:28/08:30:01-08:30:02/08:32-08:39/08:42/08:44-08:45, 11:67, 14:15	DR8, -, Undefined	215 bp
Mix 11	*08:01:01-08:01:03/08:01:05-08:03:02/08:05?/08:07/08:08?/08:09/08:11/08:13-08:16/08:17?/08:18-08:19/08:21/08:23-08:27/08:29-08:30:02/08:32-08:38/08:41, 12:16/12:22, 14:68/14:93	DR8, Undefined, -, DR14	250 bp
Mix 12	*08:04:01-08:04:02/08:04:04w/08:04:05-08:04:07/08:06/08:10/08:12/08:22w/08:28/08:31, 11:67, 12:01:01-12:15/12:17-12:19/12:21/12:23-12:27, 13:17/13:116, 14:04/14:11/14:15/14:28/14:31/14:50/14:52/14:71/14:73/14:76/14:79/14:107, DRB4*01:02?	DR8, -, DR8/DR11, DR12, Null, DR13, DR1404/DR14, Undefined	250 bp
Mix 13	*08:08/08:15/08:31, 12:04, 14:04/14:11/14:28/14:31/14:50/14:68/14:71/14:73/14:79/14:93/14:107	DR8, DR8/DR11, DR12, DR1404/DR14, -	170 bp
Mix 14	*08:14, DRB8*01:01?	DR8, -	205 bp
Mix 15	*08:16	DR8	105 bp
Mix 16	*08:17/08:28/08:37/08:45, 11:67, 12:01:01-12:19/12:22-12:27, 13:17	DR8, -, DR12, Null, DR13	135 bp

Mix	Amplified Alleles	Serology	Product Size
Mix 17	*08:21, DRB8*01:01?	DR8, –	205 bp
Mix 18	*08:23	DR8	130 bp
Mix 19	*08:26, 12:13, DRB8*01:01?	DR8, DR12, –	165 bp
Mix 20	*08:27, DRB8*01:01?	DR8, –	195 bp
Mix 21	*12:07	DR12	200 bp
Mix 22	*04:04:05/04:05:08/04:47/04:53/04:82, 08:25, 11:01:03/11:01:10-11:01:11/11:04:07/11:19:02, 12:08/12:27, 13:02:02/13:26/13:60/13:77, 15:50N, 16:01:01/16:02:01/16:03/16:05:01-16:05:02/16:07-16:17, DRB5*01:01:01/01:02-01:03/01:05/01:07-01:10N/01:13-01:14	–, DR4, DR8, DR11, DR12, DR13, DR14, DR6, Null, DR16, DR51, Undefined	180 bp
Mix 23	*12:03:02/12:19, DRB3*02:01w/02:04w, DRB3*03:02w, DRB8*01:01?	DR12, –, DR52, Undefined	205 bp
Mix 24	Negative Control		none (440 bp)

Table 2: Amplification patterns of HLA-DRB1*08, HLA-DRB1*12 and other HLA-DRB alleles detected by the HLA-DRB1*08/12 SUPTYPING CTS-PCR-SSP primer mixes (**Lot No. DR0812L03-1**) based on the IMGT/HLA Sequence Database Release 3.4.0, April 2011

Alleles	Serology	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
*04:04:05/04:05:08/04:47/04:53/04:82, 11:01:03/11:01:10-11:01:11/11:04:07/11:19:02, 13:02:02/13:26/13:60/13:77, 15:50N, 16:01:01/16:02:01/16:03/16:05:01-16:05:02/16:07- 16:17, DRB5*01:01:01/01:02-01:03/01:05/01:07- 01:10N/01:13-01:14	-, DR4, DR11, DR13, DR14, DR6, Null, DR16, DR51, Undefined																						22	
*08:01:01-08:01:03/08:01:05	DR8					5				9	10	11												
*08:01:04/08:39	DR8, -					5				9	10													
*08:02:01-08:02:03	DR8					5	6				10	11												
*08:03:02/08:33/08:36/08:38	DR8, -				4					9	10	11												
*08:04:01-08:04:02/08:04:05/08:04:07	DR8, -					5	6				10		12											
*08:04:03/08:42/08:44	DR8, -					5	6				10													
*08:04:04	DR8					5	6				10		w											
*08:04:06	-					5					10		12											
*08:05	DR8					5			8	9		?												
*08:06	DR8					5				9	10		12											
*08:07	DR8		2			5					10	11												
*08:08	DR8					5					10	?		13										
*08:09	DR8	1				5	6	w			10	11												
*08:10	DR8				4					9	10		12											
*08:11	DR8					5					10	11												
*08:12	DR8		2		4					9	10		12											
*08:13	DR8						6				10	11												
*08:14	DR8				4					9	10	11			14									
*08:15	DR8				4		6				10	11		13										
*08:16	DR8					5				9	10	11				15								
*08:17	DR8					5				9	10	?					16							
*08:18	DR8				4				8	9		11												
*08:19/08:30:02/08:34	DR8, -				4						10	11												
*08:20, 11:23/11:25/11:45/11:55/11:64, 13:13/13:18/13:47/13:55, 14:03:01- 14:03:02/14:12:01- 14:12:02/14:27/14:63/14:67/14:78/14:85/14:89/14:102	undefined, DR11, -, DR13, DR1403/DR14, DR6										10													
*08:21	DR8	1				5	6	w			10	11						17						
*08:22	DR8		w			5				9	10		w											
*08:23	DR8				4					9	10	11								18				

Alleles	Serology	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
*08:24/08:41	DR8, -					5	6		8			11												
*08:25	DR8				4				8			11											22	
*08:26	DR8					5				9	10	11								19				
*08:27	DR8				4					9	10	11									20			
*08:28, 11:67	DR8, -					5	6				10		12				16							
*08:29	DR8				4					9		11												
*08:30:01	DR8				4		6				10	11												
*08:31	DR8/DR11					5	6		8				12	13										
*08:32/08:35	Undefined, -	1			4					9	10	11												
*08:37	-				4					9	10	11					16							
*08:40	-								8	9														
*08:43	-					5				9														
*08:45	-				4		6				10						16							
*11:13:01-11:13:02/11:17/11:52, 13:76, 14:01:01/14:05:01-14:05:02/14:07:01- 14:08/14:10/14:14/14:23:01- 14:23:03/14:26/14:32:02/14:34-14:36/14:39/14:43- 14:44:01/14:45/14:54/14:56/14:60- 14:62/14:65/14:72/14:82/14:86- 14:88/14:90/14:96/14:99-14:101/14:104- 14:105/14:110-14:113	DR11, DR14, DR13, DR6, -	1																						
*12:01:01/12:06/12:10	DR12	1	2	3	4				8				12				16							
*12:01:02	DR12		2		4				8				12				16							
*12:01:03	DR12		2	3					8				12				16							
*12:02:01	DR12	1	2	3		5			8				12				16							
*12:02:02	DR12		2	3		5			8				12				16							
*12:02:03	DR12		2	3									12				16							
*12:02:04	DR12	1	2			5							12				16							
*12:02:05/12:17-12:18/12:23-12:25	-, Null	1	2	3									12				16							
*12:03:02	DR12			3	4				8				12				16							23
*12:04	DR12	1	2	3	4		6		8				12	13			16							
*12:05	DR12	1	2	w	4			7	8				12				16							
*12:07	DR12	1	2	3	4				8				12				16					21		
*12:08	DR12	1	2	3	4				8				12				16						22	
*12:09	DR12	1	2	3	4		6		8				12				16							
*12:11	DR12	1	2	3	4	5			8				12				16							
*12:12	DR12	1	2	3					8				12				16							
*12:13	DR12	1	2	3		5			8				12				16			19				
*12:14/12:26	DR12, -		2										12				16							
*12:15	DR12		2					7					12				16							
*12:16	-					5			8			11					16							
*12:19	-	1		3									12				16							23
*12:20	-	1		3																				
*12:21	-	1	2	3									12											
*12:22	-											11					16							
*12:27	-	1	2	3									12				16						22	
*13:17	DR13				4		6		8				12				16							

Alleles	Serology	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
*13:116, 14:52	-, DR14						6		8				12											
*14:04	DR1404/DR14	1						w					12	13										
*14:11	DR14	1					6	w					12	13										
*14:15	DR8	1				5	6	w			10		12											
*14:28	DR14	1	2					w					12	13										
*14:31	DR14	1						w	8				12	13										
*14:40/14:77/14:84	DR14, -	1									10													
*14:50	DR14							w					12	13										
*14:55	DR14	1									w													
*14:68	DR14	1										11		13										
*14:71/14:107	-	1											12	13										
*14:73	-	1				5							12	13										
*14:76	-												12											
*14:79	-												12	13										
*14:93	-											11		13										
DRB3*02:01/02:04, DRB3*03:02	DR52, Undefined																							w
DRB4*01:02	Undefined												?											
DRB8*01:01	-	?													?			?		?	?			?

w = weak

? = nucleotide sequence information not available for primer matching