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Manual No.: 16
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Product No.: 216
Lot No.: DR16L05-1

CTS *Collaborative Transplant Study*

WORKING INSTRUCTION
HLA-DRB1*16 SUBTYPING
CTS-PCR-SSP MINITRAY KIT

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following products:

Product No.	Description	
216	HLA-DRB1*16 Subtyping CTS-PCR-SSP Minitray Kit	  0197

Main differences between Lot DR16L05-1 (the current lot) and Lot DR16L05-0:

- 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 high resolution typing of HLA-DRB1*16 by the PCR-SSP method.
- Allele coverage: IMGT/HLA Sequence Database 3.4.0, April 2011.
- This manual is only valid for Lot No. **DR16L05-1**.
- This manual should be used together with the Main Manual (General Information Sheet) which is 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): 13 (12 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, yellow PCR-minitrays.
- PCR buffer: 1 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 Tables (Table 2 and 3) to make the 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 also 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 No.12 detects nearly all DRB1*16 except DRB1*1604, DRB1*1612 and DRB1*1618.

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-DRB1*16 SUBTYPING CTS-PCR-SSP primer mix (Lot No. DR16L05-1) based on the IMGT/HLA Sequence Database 3.4.0, April 2011

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

Primer Mix	Allele specificity	Serology	Product Size
Mix 1	*16:01:01-16:01:02/16:03w/16:04/16:08-16:09/16:13N/16:15	DR16, Null, –	200 bp
Mix 2	*16:02:01-16:02:02/16:10-16:11/16:14/16:16-16:18	DR16, –	200 bp
Mix 3	*16:03	DR16	220 bp
Mix 4	*04:12/04:18/04:25/04:31/04:54-04:55/04:58/04:86, 08:01:01-08:04:07/08:06-08:16/08:19-08:23/08:26-08:27/08:30:01-08:30:02/08:32-08:36/08:38-08:39/08:42/08:44, 13:13/13:47/13:55, 14:03:01-14:03:02/14:12:01-14:12:02/14:15/14:27/14:40/14:63/14:67/14:77-14:78/14:84-14:85/14:89/14:102, 16:04/16:18, DRB5*01:04	DR4, –, DR8, undefined, DR13, DR1403/DR14, DR6, DR16	120 bp
Mix 5	*15:21, 16:05:01-16:05:02/16:07	DR2, DR16	200 bp
Mix 6	*16:07	DR16	160 bp
Mix 7	*16:08	DR16	110 bp
Mix 8	*15:01:01:01-15:06/15:08/15:09w/15:10/15:12-15:27/15:29-15:33/15:35-15:46/15:49-15:57, 16:09-16:10	DR15, –, DR2, Null, DR16	145 bp
Mix 9	*16:11	DR16	200 bp
Mix 10	*16:12	–	200 bp
Mix 11	*16:13N	Null	155 bp
Mix 12	*15:01:01:01-15:01:13/15:01:15-15:20/15:22-15:24/15:26-15:27/15:29-15:36/15:38-15:56, 16:01:01-16:03/16:05:01-16:05:02/16:07-16:11/16:13N-16:17	DR15, –, DR2, Null, DR16	225 bp
Mix 13	Negative Control	–	none (440 bp)

w = weak

Table 2: Amplification patterns of HLA-DRB1*16 alleles detected by the HLA-DRB1*16 SUBTYPING CTS-PCR-SSP primer mixes (Lot No. DR16L05-1) based on the IMGT/HLA Sequence Database 3.4.0, April 2011

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12
*16:01:01-16:01:02/16:15	DR16, –	1											12
*16:02:01-16:02:02/16:14/16:16-16:17	DR16, –		2										12
*16:03	DR16	w		3									12
*16:04	DR16	1			4								
*16:05:01-16:05:02	DR16					5							12
*16:07	DR16					5	6						12
*16:08	DR16	1						7					12
*16:09	DR16	1							8				12
*16:10	DR16		2						8				12
*16:11	DR16		2							9			12
*16:12	–										10		
*16:13N	Null	1										11	12
*16:18	–		2		4								

w = weak

Table 3: Amplification patterns of HLA-DRB1*16 and other HLA-DRB alleles detected by the HLA-DRB1*16 SUBTYPING CTS-PCR-SSP primer mixes (Lot No. DR16L05-1) based on the IMGT/HLA Sequence Database 3.4.0, April 2011

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12
*04:12/04:18/04:25/04:31/04:54-04:55/04:58/04:86, 08:01:01-08:04:07/08:06-08:16/08:19-08:23/08:26-08:27/08:30:01-08:30:02/08:32-08:36/08:38-08:39/08:42/08:44, 13:13/13:47/13:55, 14:03:01-14:03:02/14:12:01-14:12:02/14:15/14:27/14:40/14:63/14:67/14:77-14:78/14:84-14:85/14:89/14:102, DRB5*01:04	DR4, -, DR8, undefined, DR13, DR1403/DR14, DR6				4								
*15:01:01:01-15:01:13/15:01:15-15:06/15:08/15:10/15:12-15:20/15:22-15:24/15:26-15:27/15:29-15:33/15:35-15:36/15:38-15:46/15:49-15:56	DR15, -, DR2, Null								8				12
*15:01:14/15:25/15:37/15:57	-								8				
*15:07/15:11/15:34/15:47-15:48	DR15, -												12
*15:09	DR15								w				12
*15:21	DR2					5			8				
*16:01:01-16:01:02/16:15	DR16, -	1											12
*16:02:01-16:02:02/16:14/16:16-16:17	DR16, -		2										12
*16:03	DR16	w		3									12
*16:04	DR16	1			4								
*16:05:01-16:05:02	DR16					5							12
*16:07	DR16					5	6						12
*16:08	DR16	1						7					12
*16:09	DR16	1							8				12
*16:10	DR16		2						8				12
*16:11	DR16		2							9			12
*16:12	-										10		
*16:13N	Null	1										11	12
*16:18	-		2		4								

w = weak