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CTS *Collaborative Transplant Study*

WORKING INSTRUCTION

HLA-DRB1*01 SUBTYPING CTS-PCR-SSP Minitray Kit

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following products:

Product No.	Description
207	HLA-DRB1*01 Subtyping CTS-PCR-SSP Minitray Kit  

CHANGES COMPARED TO LOT DR01L04-0:

- An update with 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*01 by the PCR-SSP method.
- Allele coverage: IMGT/HLA Sequence Database Release 3.4.0, April 2011, except for HLA-DRB1*01:22 and DRB1*01:33N, which are considered to be rare and can be detected by e. g. sequencing with the CTS-SEQUENCE KIT (you may contact us for further information).
- This manual is only valid for **Lot No. DR01L04-1**.
- This manual should be used together with the Main Manual (General Information) the 'Working Instruction for the CTS-PCR-SSP MINITRAY KITS' (Manual No. 100A).

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1. Kit Composition

MINITRAY KIT

- Number of PCR primer mixes per test (per minitray): 13 (12 allele-specific and 1 negative control mix)
- Number of tests (minitrays) per kit: 12
- The primer mixes are aliquoted and lyophilized in thin-walled, green PCR-minitrays.
- PCR buffer: 0.6 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 Table (Table 2 and 3) to make allele assignment. Alternatively, you can use the SCORE Software (www.IHWG.org) for detailed result interpretation.

5. Interpretation Hints

Mix 12 occasionally causes weak non-specific reactions which can be readily recognized as such as they are faint.

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.

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: Sizes of the PCR products and allele specificities of each HLA-DRB1*01 SUBTYPING CTS-PCR-SSP primer mix (Lot No. DR01L04-1) based on IMGT/HLA Sequence Database Release 3.4.0, April 2011

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

Primer Mix	Allele Specificity	Serology	Product Size (bp)
Mix 1	*01:01:01-01:01:20/01:03/01:05/01:07-01:19/01:21/01:24-01:25/01:27-01:32/01:36	DR1, –, DR103/DR1	255
Mix 2	*01:01:01-01:01:14/01:01:16-01:02:05/01:04-01:05/01:07-01:08/01:11-01:14/01:18/01:20-01:21/01:24-01:32/01:34-01:36	DR1, –	210
Mix 3	*01:02:01-01:02:05/01:23/01:26/01:34	DR1, –	235
Mix 4	*01:03	DR103/DR1	210
Mix 5	*01:04/01:11/01:35	DR1, –	230
Mix 6	*01:05	DR1	130
Mix 7	*01:06/01:09/01:15	DR1	210
Mix 8	*01:07	DR1	270
Mix 9	*01:08	DR1	110
Mix 10	*01:10	DR1	210
Mix 11	*01:12	DR1	215
Mix 12	*01:13	DR1	165
Mix 13	Negative Control	–	none (440)

Table 2: Amplification patterns of HLA-DRB1*01 alleles detected by HLA-DRB1*01 SUBTYPING CTS-PCR-SSP primer mixes (**Lot No. DR01L04-1**) based on IMGT/HLA Sequence Database Release 3.4.0, April 2011

Allele	Serology	1	2	3	4	5	6	7	8	9	10	11	12
*01:01:01-01:01:14/01:01:16-01:01:20/01:14/01:18/01:21/01:24-01:25/01:27-01:32/01:36	DR1, –	1	2										
*01:01:15/01:16-01:17/01:19	DR1, –	1											
*01:02:01-01:02:05/01:26/01:34	DR1, –		2	3									
*01:03	DR103/DR1	1			4								
*01:04/01:35	DR1, –		2			5							
*01:05	DR1	1	2				6						
*01:06	DR1							7					
*01:07	DR1	1	2						8				
*01:08	DR1	1	2							9			
*01:09/01:15	DR1	1						7					
*01:10	DR1	1									10		
*01:11	DR1	1	2			5							
*01:12	DR1	1	2									11	
*01:13	DR1	1	2										12
*01:20	–		2										
*01:23	–			3									