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Manual No.: 07
Revision: February 7, 2008
Product No.: 207 + 407
Lot No.: DR01L04-0

CTS Collaborative Transplant Study

WORKING INSTRUCTION
HLA-DRB1*01 SUBTYPING
CTS-PCR-SSP Minitrays

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 # 003:

- The kit has thoroughly been revised to cover all new alleles.
- The total number of primer mixes has been increased from 8 to 13 (12 allele-specific mixes and 1 negative control mix).
- Mixes number **9 to 12** are new allele-specific mixes.

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 2.12.0, January 2006.
- This manual is only valid for **Lot No. DR01L04-0**.
- 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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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) 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. DR01L04-0) based on IMGT/HLA Sequence Database Release 2.12.0, January 2006

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

| Primer mix | Allele specificity | Serology | Product sizes (bp) |
|------------------|------------------------------------|---------------|--------------------|
| Mix 1 | DRB1*010101-0103/03/05/07-13 | DR1, DR103, - | 255 |
| Mix 2 | DRB1*010101-0204/04/05/07/08/11-13 | DR1, - | 210 |
| Mix 3 | DRB1*010201-0204 | DR1 | 235 |
| Mix 4 | DRB1*0103 | DR103 | 210 |
| Mix 5 | DRB1*0104/11 | DR1, - | 230 |
| Mix 6 | DRB1*0105 | - | 130 |
| Mix 7 | DRB1*0106/09 | - | 210 |
| Mix 8 | DRB1*0107 | - | 270 |
| Mix 9 | DRB1*0108 | - | 110 |
| Mix 10 | DRB1*0110 | - | 210 |
| Mix 11 | DRB1*0112 | - | 215 |
| Mix 12 | DRB1*0113 | - | 165 |
| Negative Control | - | - | - |

Table 2: Amplification patterns of HLA-DRB1*01 alleles detected by HLA-DRB1*01 SUBTYPING CTS-PCR-SSP primer mixes (**Lot No. DR01L04-0**) based on IMGT/HLA Sequence Database Release 2.12.0, January 2006

| | Mix No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-------------|----------|---|---|---|---|---|---|---|---|---|----|----|----|
| Allele | Serology | | | | | | | | | | | | |
| DRB1*010101 | DR1 | + | + | | | | | | | | | | |
| DRB1*010102 | DR1 | + | + | | | | | | | | | | |
| DRB1*010103 | DR1 | + | + | | | | | | | | | | |
| DRB1*010201 | DR1 | | + | + | | | | | | | | | |
| DRB1*010202 | DR1 | | + | + | | | | | | | | | |
| DRB1*010203 | DR1 | | + | + | | | | | | | | | |
| DRB1*010204 | DR1 | | + | + | | | | | | | | | |
| DRB1*0103 | DR103 | + | | | + | | | | | | | | |
| DRB1*0104 | DR1 | | + | | | + | | | | | | | |
| DRB1*0105 | - | + | + | | | | + | | | | | | |
| DRB1*0106 | - | | | | | | | + | | | | | |
| DRB1*0107 | - | + | + | | | | | | + | | | | |
| DRB1*0108 | - | + | + | | | | | | | + | | | |
| DRB1*0109 | - | + | | | | | | + | | | | | |
| DRB1*0110 | - | + | | | | | | | | | + | | |
| DRB1*0111 | - | + | + | | | + | | | | | | | |
| DRB1*0112 | - | + | + | | | | | | | | | + | |
| DRB1*0113 | - | + | + | | | | | | | | | | + |