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Manual No.:	40
Revision:	May 4, 2011
Product No.:	340
Lot No.:	SEC04L01-1

CTS *Collaborative Transplant Study*


WORKING INSTRUCTION

HLA-C*04:09N
CTS-PCR-SSP Minitray Kit

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following product:

Product No.	Description
340	HLA-C*04:09N CTS-PCR-SSP Minitray Kit



Introduction

- Intended use: This kit allows the distinction of HLA-C*04:09N from other alleles of the HLA-C*04 allele group by the PCR-SSP method. HLA-C*04:09N belongs to the HLA-C*04:01:01G group. All alleles of this group have the same nucleotide sequence in exon 2 and exon 3. HLA-C*04:09N exhibits a deletion in exon 7 which is unique among the HLA-class I alleles.
- Allele coverage: IMGT/HLA Sequence Database Release 3.4.0, April 2011
- This manual is only valid for **Lot No. SEC04L01-1** 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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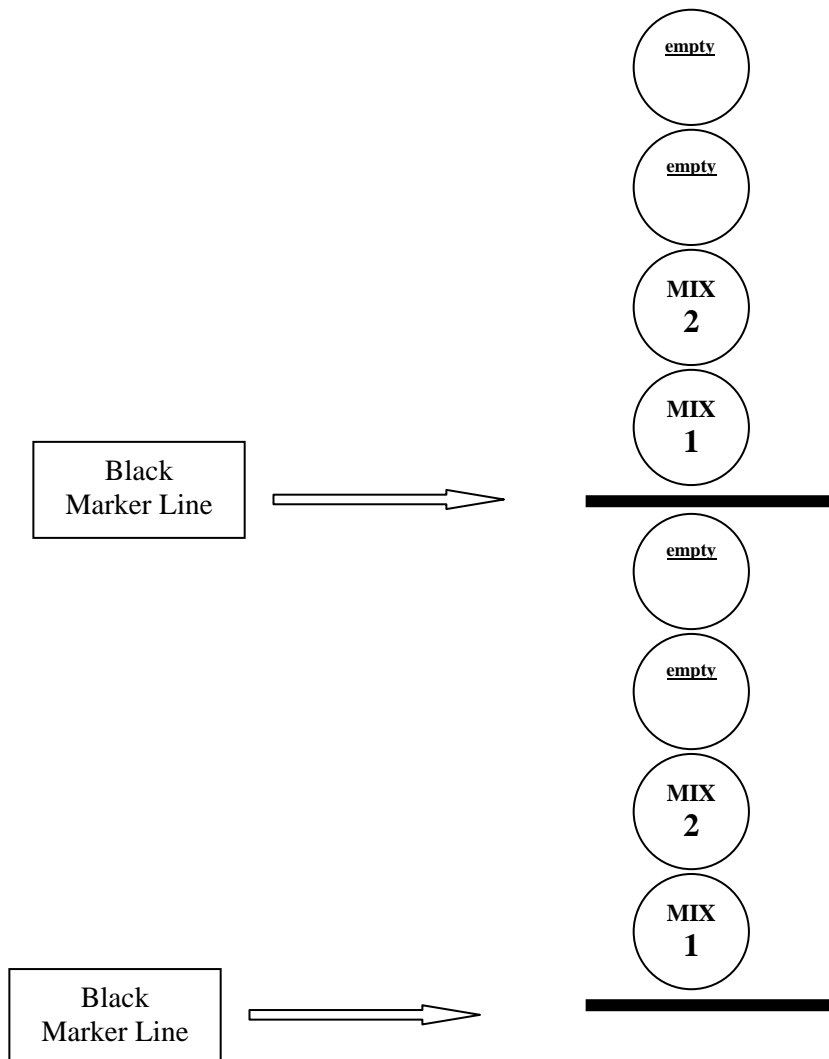
1. Kit Composition

8-well Minitray Kit

- Number of PCR primer mixes per test: 2 allele-specific mixes (see Fig. 1)
- Number of tests per Minitray: 2
- Number of tests per kit: 24 (12 Minitrays)
- The primer mixes are aliquoted and lyophilized in thin-walled, red PCR-Minitrays.
- PCR buffer: 0.3 ml of 7.5% Mastermix (without Taq polymerase)

Figure 1.

HLA-C*04:09N CTS-PCR-SSP Minitray Kit: Mix position in an 8-well Minitray



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.

For one typing, please use the following:

Set up:

5,75	µl	Mastermix
13,7	µl	H2O
2,0	µl	DNA
0,12	µl	Taq

4. Result Evaluation

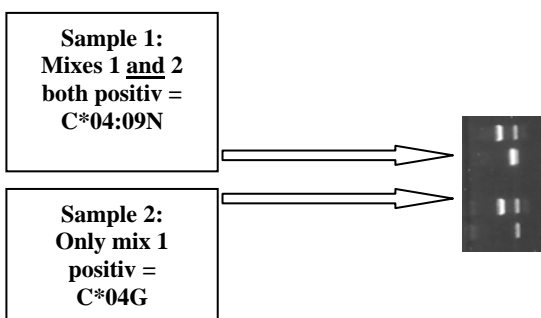
Check the approximate size of the PCR product against the Primer Mix Specificity Table (Table 1) to confirm the correct product size.

SPECIAL NOTES

If only mix 1 of this kit is positive, the DNA is typed negative for C*04:09N (no C*04:09N detectable).

In contrast, if mix 1 and 2 are both positive, the DNA is typed positive for HLA-C*04:09N (C*04:09N present).

Example:



Please note that the position of the allele-specific fragment of mix 2 (only 138 bp) is close to the amplification control (89 bp)!

- The HLA-C alleles listed in Table 1 and 2 (except HLA-C*04G) can be distinguished by their amplification patterns of the primer mixes provided by the HLA-C CTS-PCR-SSP Tray Kit.
- HLA-C*04G and HLA-C* 04:09N generate the **same** amplification pattern with the primer mixes of HLA-C Typing CTS-PCR-SSP Tray Kit, Lot C10-0, mixes 6 and 7 positive.

The priming sites of the specific primers of mix 7 are located outside of exons 2 and 3 of the HLA-C alleles. For many HLA-Class I variants, only the sequences of the antigen recognition sites (exons 2 and 3) are reported. Even though the HLA-C*04:09N CTS-PCR-SSP Minitray Kit has been extensively tested and validated, a cross reaction with a rare or new allele due to mutations in the priming sites cannot be categorically ruled out.

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.

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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E-mail: hien.tran@med.uni-heidelberg.de

Hien Tran, M.D.

Table 1: Sizes of the PCR products and allele specificities of each primer mix of the HLA-C*04:09N CTS-PCR-SSP Minitray Kit (Lot SEC04L01-1) are based on the IMGT/HLA Sequence Database Release 3.4.0, April 2011

Amplification Control (Internal Positive Control): product size 89 base pairs (bp)

Primer mix	Allele specificity	Product size (bp)
Mix 1	04:01:01:01-04:01:22/04:01:24-04:01:29/04:01:31/04:03-04:08/04:10-04:14/04:15:02-04:20/04:23-04:36/04:38-04:72/04:74-04:79/04:81-04:82/04:84-04:90, *02:02:11, 08:02:06/08:19, 12:10:01/12:31, B*37:01:06, B*67:02	470
Mix 2	C*04:09N	138

Table 2: Amplification patterns of HLA-C alleles detected by the HLA-C*04:09N CTS-PCR-SSP Minitray Kit (Lot SEC04L01-1) based on the IMGT/HLA Sequence Database Release 3.4.0, April 2011

Allele	Mix 1	Mix 2
C*02:02:11	1	
C*04:01:01:01	1	
C*04:01:01:02	1	
C*04:01:01:03	1	
C*04:01:01:04	1	
C*04:01:02	1	
C*04:01:03	1	
C*04:01:04	1	
C*04:01:05	1	
C*04:01:06	1	
C*04:01:07	1	
C*04:01:08	1	
C*04:01:09	1	
C*04:01:10	1	
C*04:01:11	1	
C*04:01:12	1	
C*04:01:13	1	
C*04:01:14	1	
C*04:01:15	1	
C*04:01:16	1	
C*04:01:17	1	
C*04:01:18	1	
C*04:01:19	1	
C*04:01:20	1	
C*04:01:21	1	
C*04:01:22	1	
C*04:01:24	1	
C*04:01:25	1	
C*04:01:26	1	
C*04:01:27	1	
C*04:01:28	1	
C*04:01:29	1	
C*04:01:31	1	
C*04:03	1	
C*04:04:01	1	
C*04:04:02	1	
C*04:05	1	
C*04:06	1	
C*04:07	1	
C*04:08	1	
C*04:09N	1	2
C*04:10	1	
C*04:11	1	
C*04:12	1	
C*04:13	1	
C*04:14	1	
C*04:15:02	1	
C*04:15:03	1	
C*04:16	1	
C*04:17	1	
C*04:18	1	
C*04:19	1	
C*04:20	1	
C*04:23	1	
C*04:24	1	
C*04:25	1	
C*04:26	1	
C*04:27	1	
C*04:28	1	
C*04:29	1	
C*04:30	1	
C*04:31	1	
C*04:32	1	
C*04:33	1	
C*04:34	1	
C*04:35	1	
C*04:36	1	
C*04:38	1	
C*04:39	1	
C*04:40	1	
C*04:41	1	
C*04:42	1	
C*04:43	1	
C*04:44	1	
C*04:45	1	

Allele	Mix 1	Mix 2
C*04:46	1	
C*04:47	1	
C*04:48	1	
C*04:49	1	
C*04:50	1	
C*04:51	1	
C*04:52	1	
C*04:53	1	
C*04:54	1	
C*04:55	1	
C*04:56	1	
C*04:57	1	
C*04:58	1	
C*04:59Q	1	
C*04:60	1	
C*04:61	1	
C*04:62	1	
C*04:63	1	
C*04:64	1	
C*04:65	1	
C*04:66	1	
C*04:67	1	
C*04:68	1	
C*04:69	1	
C*04:70	1	
C*04:71	1	
C*04:72	1	
C*04:74	1	
C*04:75	1	
C*04:76	1	
C*04:77	1	
C*04:78	1	
C*04:79	1	
C*04:81	1	
C*04:82	1	
C*04:84	1	
C*04:85	1	
C*04:86	1	
C*04:87	1	
C*04:88N	1	
C*04:89	1	
C*04:90	1	
C*08:02:06	1	
C*08:19	1	
C*12:10:01	1	
C*12:31	1	
B*37:01:06	1	
B*67:02	1	

Worksheet for the identification of C*04:09N

HLA-C*04:09N CTS-PCR-SSP Minitray Kit

DNA-No.: _____

Set up:

5,75 µl Mastermix
13,7 µl H2O
2,0 µl DNA
0,12 µl Taq

Amplification Control 90bp

Gel Photo	Mix 1	04:01:01:01-04:01:22/04:01:24-04:01:29/04:01:31/04:03-04:08/04:10-04:14/04:15:02-04:20/04:23-04:36/04:38-04:72/04:74-04:79/04:81-04:82/04:84-04:90, *02:02:11, 08:02:06/08:19, 12:10:01/12:31, B*37:01:06, B*67:02	470bp
	Mix 2	C*04:09N	138bp

Result:

<u>No</u> C*04:09N	
C*04:09N <u>positive</u>	

Date: _____

Technician: _____

Lab. Supervisor: _____