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Lot No.	SEC04L03-3

**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 

#### 1. Main differences

- **Between Lot SEC04L03-3 (the current lot) and Lot SEC04L03-2:**  
The kit was updated to cover new alleles included in the IMGT/HLA Database of January 2017. Deleted and renamed alleles were taken into consideration.

#### 2. 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.
- This manual is only valid for **Lot No. SEC04L03-3**.
- 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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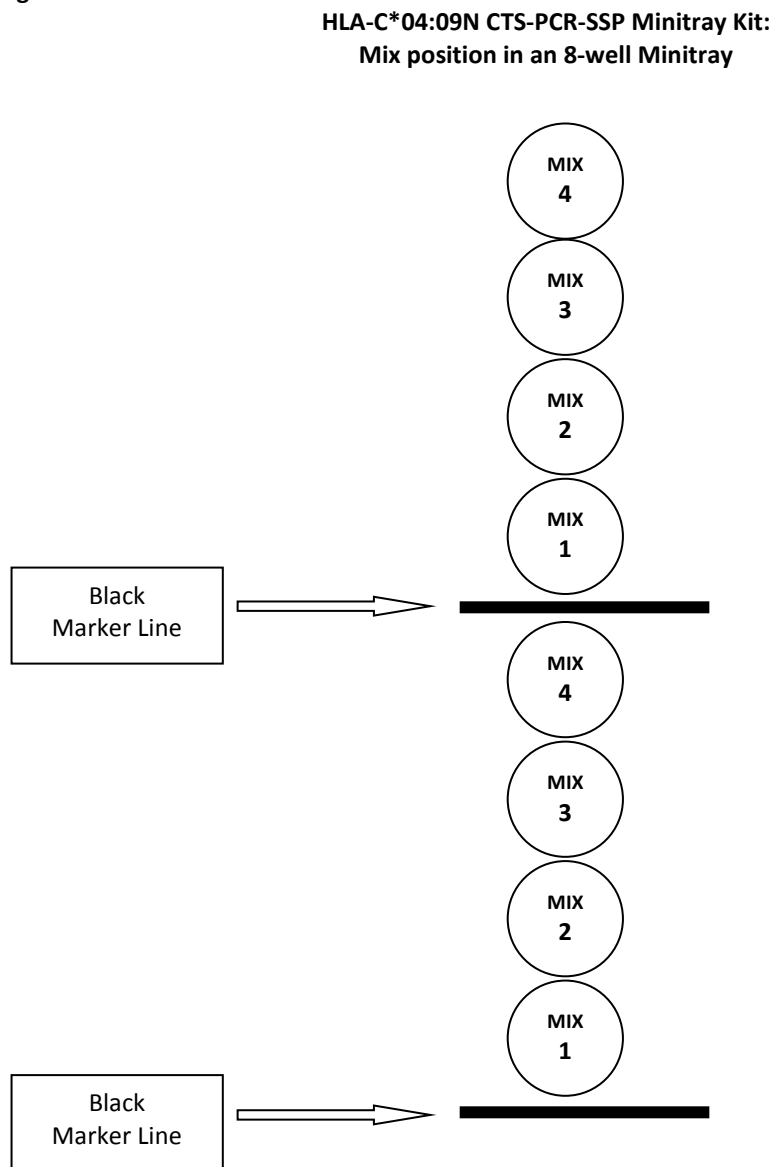
Table 1: Sizes of the PCR products and allele specificities of each primer mix of the HLA-C*04:09N CTS-PCR-SSP Minitray Kit (Lot SEC04L03-3) based on the IMGT/HLA Sequence Database Release 3.27.0, January 2017 .....	6
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Table 2: Amplification patterns of HLA-C*04:01/04:09N by the HLA-C*04:09N CTS-PCR-SSP primer mixes (Lot SEC04L03-3) based on IMGT/HLA Sequence Database Release 3.27.0, January 2017 .....	7
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#### 4. Kit Composition

- 8-well Minitray Kit
- Number of PCR primer mixes per test: 4 allele-specific mixes (see Figure 1)
- Number of tests per Minitray: 2
- Number of tests per kit: 24 (12 Minitrays)
- The primer mixes are aliquoted and dried in thin-walled, red PCR-Minitrays.
- PCR buffer: 0.3 ml of Mastermix SSP (without Taq polymerase)

Figure 1.



#### 5. 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.

## 6. 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.



## 7. Result Evaluation

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

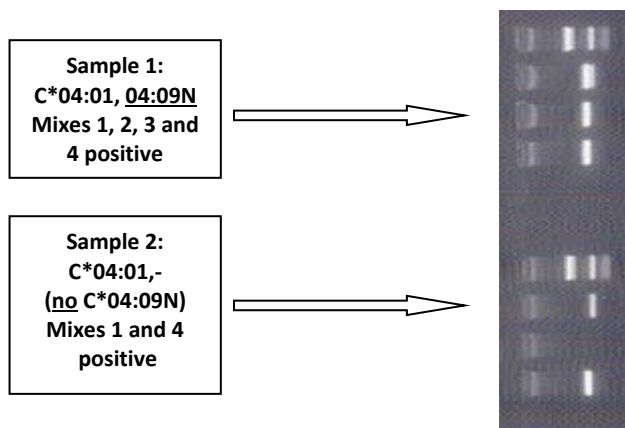
## 8. Interpretation Hints

- 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.

## 9. Special notes

- For the detection of HLA-C\*04:09N in a sample which is ambiguous for HLA-C\*04:01/04:09N:
- If HLA-C\*04:09N is present, mixes 1, 2, and 3 are positive. If the second allele is e. g. HLA-C\*04:01, mix 4 would also be positive (see example, sample 1).
- In contrast, if no C\*04:09N is present, HLA-C\*04:01 will be amplified by mixes 1 and 4 whereas mixes 2 and 3 remain negative (see example, sample 2).

### Example:



- The allele-specific fragment of mix 2 is **small** (only 140 bp) and lies in proximity to the amplification control (90 bp)!
- Mixes 3 and 4 contain **no** amplification control!
- 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 C12-1, 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.

#### **10. 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.

#### **11. 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.

#### **12. 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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Fax: +49 6221 564200

E-mail: [hien.tran@med.uni-heidelberg.de](mailto:hien.tran@med.uni-heidelberg.de)

Hien Tran, M.D.

### 13. Appendix

**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 SEC04L03-3**) based on the IMGT/HLA Sequence Database Release 3.27.0, January 2017

Mix	Allele	Serology	Size
Mix 1	C*02:02:11,04:01:01:01-04:01:22/04:01:24-04:01:29/04:01:31-04:01:81/ 04:03:01-04:14/04:15:02-04:20/04:23-04:36/04:38-04:79/04:81-04:99/ 04:101-04:117/04:119-04:129/04:131-04:177/04:179-04:186/04:187w/ 04:188-04:196/04:198-04:263,05:01:20/05:64:01,08:02:06/08:19/08:62/ 08:144,12:02:09/12:10:01/12:31/12:96,15:78:02,16:104,B*08:01:36, B*37:01:06,B*67:02	Cw2, Cw4, -, Null, B37	475 bp
Mix 2	C*04:09N	Null	140 bp
Mix 3	C*04:09N	Null	140 bp
Mix 4	C*01:02:01/01:02:11/01:02:29-01:02:30/01:02:36/01:03:01:06/01:08:01:14/ 01:21/01:30/01:40/01:67/01:93/01:99/01:106/01:110/01:117N,02:16:02w, 03:02:01-03:02:02:03/03:03:01:01/03:03:27-03:03:28/03:04:01:01- 03:04:01:02/03:04:02/03:04:04/03:04:48/03:05-03:06:01/03:13:01/03:20N/ 03:40:01/03:41:02/03:46/03:51/03:61/03:87:02/03:100/03:140/03:192/ 03:219/03:243/03:250-03:251/03:261/03:271/03:285-03:290,04:01:01:01- 04:01:01:06/04:01:44/04:01:62/04:01:66/04:01:68/04:03:01/04:06/04:10/ 04:13/04:15:02/04:42:02/04:70-04:71/04:128/04:145/04:155/04:161/04:177/ 04:186-04:189/04:191N/04:198-04:201,05:01:01:01-05:01:01:02/05:08- 05:09:01/05:37/05:92N-05:93/05:98/05:109-05:111/05:113N/05:117-05:118, 06:02:01:01-06:02:01:03/06:02:31/06:02:42/06:02:44/06:04:02/06:23- 06:24/06:46N/06:76:01/06:103/06:106:02/06:120/06:127:01:01/06:138/ 06:142/06:146-06:149/06:153-06:155:01:01/06:156/06:160,08:01:01/ 08:01:03/08:02:01:01-08:02:01:02/08:02:11-08:02:12/08:03:01/08:04:01/ 08:12/08:20-08:22/08:24/08:27/08:36N/08:40-08:41/08:62/08:72:02- 08:73/08:90/08:92/08:94/08:103/08:112-08:113/08:127N,12:02:02:01/ 12:02:12/12:03:01:01-12:03:01:03/12:03:29/12:03:34:01-12:03:34:02/ 12:03:36-12:03:37/12:08/12:13/12:16/12:19/12:22/12:59/12:73/12:99:01/ 12:139-12:141/12:153-12:156/12:160-12:161,14:02:01:01/14:02:12/ 14:02:17/14:02:19/14:03/14:21N/14:23/14:69/14:71,15:02:01:01- 15:02:01:03/15:05:01-15:05:02/15:13/15:16-15:17/15:96Q/15:104, 16:01:01:01-16:01:01:02/16:01:18/16:02:01/16:04:01/16:04:03/16:84, 17:01:01:02w-17:01:01:05w/17:03:01w/17:17w/17:30w,18:01-18:02/18:10	Cw1, -, Null, Cw10(w3), Cw9(w3), Cw3, Cw4, Cw5, Cw6, Cw8	140 bp

**Amplification Control (Internal Positive Control):** only in Mix 1 and Mix 2!

Product size 90 base pairs (bp)

**w** = weak

**Table 2:** Amplification patterns of HLA-C\*04:01/04:09N by the **HLA-C\*04:09N** CTS-PCR-SSP primer mixes (**Lot SEC04L03-3**) based on IMGT/HLA Sequence Database Release 3.27.0, January 2017

Allele	Serology	1	2	3	4
C*01:02:01/01:02:11/01:02:29-01:02:30/01:02:36/01:03/01:06/01:08/01:14/01:21/01:30/01:40/01:67/01:93/01:99/01:106/01:110/01:117N,03:02:01-03:02:02/03:03:01-01/03:03:27-03:03:28/03:04:01-01-03:04:01:02/03:04:02/03:04:04/03:04:48/03:05-03:06:01/03:13:01/03:20N/03:40:01/03:41:02/03:46/03:51/03:61/03:87:02/03:100/03:140/03:192/03:219/03:243/03:250-03:251/03:261/03:271/03:285-03:290,05:01:01-01-05:01:01:02/05:08-05:09:01/05:37/05:92N-05:93/05:98/05:109-05:111/05:113N/05:117-05:118,06:02:01-01-06:02:01:03/06:02:31/06:02:42/06:02:44/06:04:02/06:23-06:24/06:46N/06:76:01/06:103/06:106:02/06:120/06:127:01:01/06:138/06:142/06:146-06:149/06:153-06:155:01:01/06:156/06:160,08:01:01/08:01:03/08:02:01-01-08:02:01:02/08:02:11-08:02:12/08:03:01/08:04:01/08:12/08:20-08:22/08:24/08:27/08:36N/08:40-08:41/08:72:02-08:73/08:90/08:92/08:94/08:103/08:112-08:113/08:127N,12:02:02:01/12:02:12/12:03:01-01-12:03:01:03/12:03:29/12:03:34:01-12:03:34:02/12:03:36-12:03:37/12:08/12:13/12:16/12:19/12:22/12:59/12:73/12:99:01/12:139-12:141/12:153-12:156/12:160-12:161,14:02:01:01/14:02:12/14:02:17/14:02:19/14:03/14:21N/14:23/14:69/14:71,15:02:01:01-15:02:01:03/15:05:01-15:05:02/15:13/15:16-15:17/15:96Q/15:104,16:01:01:01-16:01:01:02/16:01:18/16:02:01/16:04:01/16:04:03/16:84,18:01-18:02/18:10	Cw1, -, Null, Cw10(w3), Cw9(w3), Cw3, Cw5, Cw6, Cw8				4
C*02:02:11,04:01:01:07-04:01:22/04:01:24-04:01:29/04:01:31-04:01:43/04:01:45-04:01:61/04:01:63-04:01:65/04:01:67/04:01:69-04:01:81/04:03:02-04:05/04:07-04:08/04:11-04:12/04:14/04:15:03-04:20/04:23-04:36/04:38-04:42:01/04:43-04:69/04:72-04:79/04:81-04:99/04:101-04:117/04:119-04:127/04:129/04:131-04:144/04:146-04:154/04:156-04:160/04:162-04:176/04:179-04:185/04:190/04:192-04:196/04:202-04:263,05:01:20/05:64:01,08:02:06/08:19/08:144,12:02:09/12:10:01/12:31/12:96,15:78:02,16:104,B*08:01:36,B*37:01:06,B*67:02	Cw2, Cw4, -, Null, B37	1			
C*02:16:02,17:01:01:02-17:01:01:05/17:03:01/17:17/17:30	-				w
C*04:01:01:01-04:01:01:06/04:01:44/04:01:62/04:01:66/04:01:68/04:03:01/04:06/04:10/04:13/04:15:02/04:42:02/04:70-04:71/04:128/04:145/04:155/04:161/04:177/04:186/04:188-04:189/04:191N/04:198-04:201,08:62	Cw4, -, Null	1			4
C*04:09N	Null	1	2	3	
C*04:187	-	w			4

w = weak

# Worksheet for the identification of C\*04:09N

Lot SEC04L03-3

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

DNA-No.: \_\_\_\_\_



Gel Photo	Mix	Specificities	Allele-specific fragment	Amplification control
	1	C*04:01/C*04:09N (see comment below)	475 bp	90 bp
	2	C*04:09N	140 bp	90 bp
	3	C*04:09N	140 bp	<b>NO</b>
	4	C*04:01 (see comment below)	140 bp	<b>NO</b>

Comment to mix 1 and mix 4: Other specificities see Manual, Table 1

### Result:

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

Date: \_\_\_\_\_

Technician: \_\_\_\_\_

Lab. Supervisor: \_\_\_\_\_