

**CTS** *Collaborative Transplant Study*

WORKING INSTRUCTION

***HUMAN MINOR HISTOCOMPATIBILITY ANTIGEN (mHA)***  
**CTS-PCR-SSP MINITRAY KIT**

**LOCUS- AND LOT-SPECIFIC MANUAL**

To be applied to the following product(s):

<b>Product No.</b>	<b>Description</b>	
229	mHA CTS- PCR-SSP Minitray Kit	<b>For research use only</b>

**Introduction**

- **Intended use:** The mHA CTS-PCR-SSP Minitray Kit provides primer mixes for the genotyping of the following human minor histocompatibility antigens (mHA): **HA-1, HA-2, HA-3, HA-8, HB-1, ACC-1, ACC-2, PANE-1** and **UGT2B17** (ref. 1 and 5). In addition, this kit contains mixes for the typing of non-synonymous SNPs (single nucleotide polymorphisms) within the coding region of the gene **PECAM-1 or CD31** (Codon 125, 563 and 670) and **LECAM-1 or CD62L** (Codon 206 and 213). There are hints that PECAM-1 (platelet endothelial cell adhesion molecule-1) and CD62L (leukocyte endothelial cell adhesion molecule-1) may encode for a mHA associated with graft-versus-host disease (ref. 2 - 4).  
 The typing technique is based on the PCR-SSP method.
- This Manual is valid for **Lot mHAL03-0** only.
- This Manual should be used together with the Main Manual (General Information) which is the 'Working Instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS' (Manual No. 100A) supplied along with this product.

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## **1. Kit Composition**

- Number of PCR primer mixes per test (one minitray): 27 allele-specific mixes + 1 negative control mix
- Number of tests (minitrays) per kit: 12
- The primer mixes are aliquoted and lyophilized in thin-walled, green 32 well-PCR minitrays.
- PCR buffer: 1,3 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).

## **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).

## **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).

## **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 Amplification Control (Internal Positive Control)**

Each of the primer mixes contains a non-allelic control primer pair which amplifies a 440 base pairs fragment of the C-reactive protein gene. Slots in which the allele-specific PCR product is present (and either no visible or only a weaker amplification control band) indicate the presence of certain allele-specific sequences (Table 1). If a tested DNA is negative for a specific mHA-allele, then this slot should only show the amplification control band.

## **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.
- It is very important to perform the electrophoresis for at least 15 min. Only by doing this, the specific amplicons will be nicely separated from the 440 bp control band. This is especially important for the larger specific products such as HA-1<sup>H</sup> and HA-1<sup>R</sup>.

- All SNPs detected by this kit are biallelic. Thus, it is theoretically possible that an individual will show positive allele-specific reactions with all PCR mixes of the kit.
- Please also refer to Section 7 of the ‘Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS’ (Manual No.: 100A).

**Special notes:** Mix 10 (HB-1Y) can show a non-specific reaction. If this occurs, the band of the non-specific product would be much weaker than the band of the specific product. The intensity of the specific band of mix 9 (HB-1H), which has usually the same intensity as the specific product of mix 10, can be used for comparison.

## **6. Troubleshooting**

Please refer to Section 8 of the ‘Working instruction for the CTS-PCR-SSP TRAY and MINITRAY KITS’ (Manual No.: 100A).

## **7. Precaution**

Please refer to the ‘Material Safety Data Sheet for the CTS-PCR-SSP TRAY and MINITRAY KITS’ (Manual No.:100B).

## **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: Mix number, fragment size and allele specificity of each PCR-SSP primer mix of the mHA CTS-PCR-SSP Minitray Kit (Lot-No. mHAL03-0)**

Mix-No.	Minor-Antigen	Polymorphism	Size of the allele-specific amplicon (base pairs)	Size of the amplification control (base pairs)
1	<i>HA-1</i>	<b>H</b>	290/295*	440
2		<b>R</b>	290/295*	440
3	<i>HA-2</i>	<b>V</b>	112	440
4		<b>M</b>	112	440
5	<i>HA-3</i>	<b>T</b>	98	440
6		<b>M</b>	99	440
7	<i>HA-8</i>	<b>R</b>	296	440
8		<b>P</b>	296	440
9	<i>HB-1</i>	<b>H</b>	141	440
10		<b>Y</b>	146	440
11	<i>ACC-1</i> ( <i>BCL2A1-1</i> )	<b>Y</b>	294	440
12		<b>C</b>	293	440
13	<i>ACC-2</i> ( <i>BCL2A1-2</i> )	<b>D</b>	104	440
14		<b>G</b>	104	440
15	<i>PECAM-1</i> ( <i>Codon 125</i> )	<b>L</b>	143	440
16		<b>V</b>	143	440
17	<i>PECAM-1</i> ( <i>Codon 563</i> )	<b>N</b>	274	440
18		<b>S</b>	273	440
19	<i>PECAM-1</i> ( <i>Codon 670</i> )	<b>R</b>	205	440
20		<b>G</b>	203	440
21	<i>CD62L</i> ( <i>Codon 206</i> )	<b>F</b>	116	440
22		<b>L</b>	116	440
23	<i>CD62L</i> ( <i>Codon 213</i> )	<b>P</b>	155	440
24		<b>S</b>	156	440
25	<i>PANE-1</i>	<b>R</b>	151	440
26		<b>Stopp</b>	151	440
27	<i>UGT2B17</i>	<b>Ex1a</b>	230	440
28	<i>Negative Control</i>	-	-	440

\*HA-1: There are 2 genetic variants known (with and without insert).

#### References:

- 1) Spierings et al., 2004. Minor histocompatibility antigens--big in tumour therapy. *Trends Immunol.* 2004 Feb;25(2):56-60. Review.
- 2) Heinemann FM et al., 2004. Impact of disparity of histocompatibility antigens HA-1, CD31, and CD49b in hematopoietic stem cell transplantation of patients with chronic myeloid leukemia with sibling and unrelated donors. *Transplantation.* Vol. 77, No. 7:1103-1106
- 3) Cavanagh G et al., 2005. Donor CD31 genotyp impacts on transplant complications after human leukocyte antigen-matched sibling allogeneic bone marrow transplantation. *Transplantation* Vol. 79, No. 5:602-605
- 4) Maruya E. et al., 1998. Evidence that CD31, CD49b, and CD62L are immunodominant minor histocompatibility antigens in HLA identical sibling bone marrow transplants. *Blood*, Vol 92, No. 6: 2169-2176
- 5) Brickner A.G. et al., 2006. The PANE1 gene encodes a novel human minor histocompatibility antigen that is selectively expressed in B-lymphoid cells and B-CLL. *May 1;107(9):3779-3786.*