

CTS *Collaborative Transplant Study*

WORKING INSTRUCTION

HUMAN PLATELET ANTIGEN (HPA)
CTS-PCR-SSP MINITRAY KIT

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following product(s):

Product No.	Description	
225	HPA CTS- PCR-SSP Minitray Kit	IVD C €

CHANGES COMPARED TO LOT HPA08-0, REVISION OF OCTOBER 28, 2009:

- **HPA-5a, 5b:** Refer to Table 1, page 6, and to the worksheet, page 8, for new size of the allele-specific PCR product.

Introduction

- **Intended use:** The HPA CTS-PCR-SSP Minitray Kit provides primer mixes for genotyping of the human platelet antigens HPA-1, -2, -3, -4, -5, -6 and -15 by the PCR-SSP method. The kit has been developed according to the publications of Kluter H et al. (Vox Sang 1996), Meyer et al. (Transfusion 1999), Lyou et al. (Transfusion 2002), and Schuh et al. (Blood 2002).
- This manual is only valid for Lot No. **HPA09-0**.
- It should be used together with the Main Manual (General Information) 'Working Instruction for the CTS-PCR-SSP Tray and Minitray Kits' (Manual No. 100A) you received along with the kit.

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1. Kit Composition

1.1. Number of tests

- Number of mixes per test (one minitray): 14 allele-specific mixes + 1 negative control mix
- Number of tests (minitrays) per kit: 12
- The primer mixes are aliquoted and lyophilized in thin-walled, nature-colored 16 well-PCR-minitrays.
- PCR buffer: 0.6ml of Mastermix HPA (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.

1.2. Positions and specificities of primer mixes

Figure 1 and Table 1 show the mix positions on the PCR-minitray and the HPA specificity for each primer mix, respectively.

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

Check the approximate base pair size of the PCR product against Table 1 to confirm the correct product size.

4.1. Amplification Control (Internal Positive Control)

Each of the primer mixes contains a non-allelic control primer pair which amplifies a 440 bp fragment of the C-reactive protein gene.

4.2. Allele-specific amplification product(s)

Slots in which the specific PCR product is present (and no visible or only a weaker amplification control band) indicate the presence of certain allele-specific sequences (Table 1).

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.
- A positive specific reaction in any of the PCR tubes indicates the presence of the corresponding HPA allele.
- 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 PCR products.
- The HPA polymorphism is based on diallelic loci within the HPA gene. Thus, it is theoretically possible that an individual will show positive 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) 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 the '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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Hien Tran, M.D.

Figure 1. HPA-mix identification and mix position on 16-well PCR-minitray

(Neg. Ctrl. = Negative Control Mix)

Size of allele-specific PCR product (basepairs = bp) is indicated for each mix.

Numbers which appear on the upper edge of the minitray can be ignored.

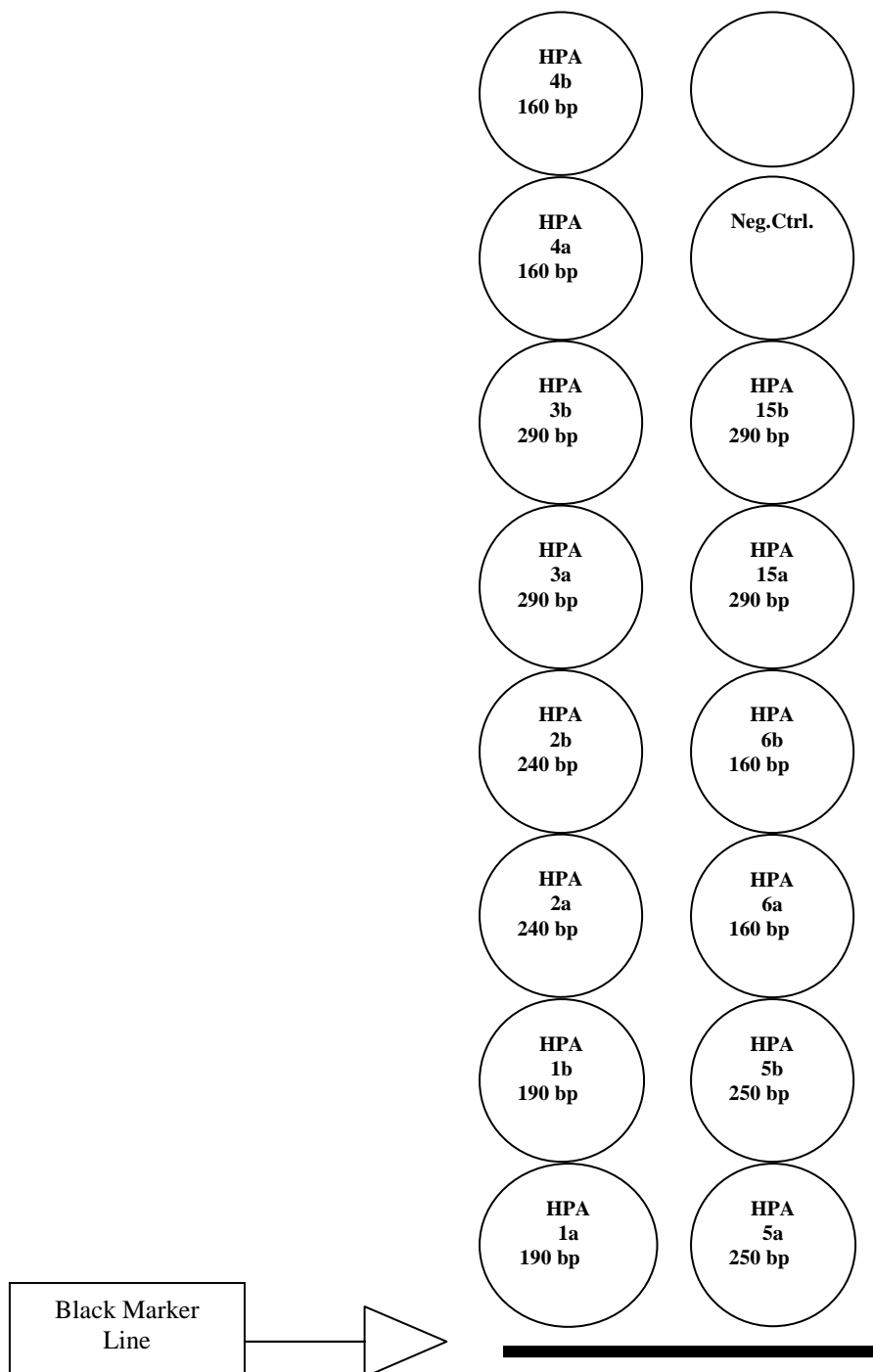


Table 1. Sizes of the PCR products and allele specificities of each PCR-SSP primer mix in the HPA CTS-PCR-SSP Minitray Kit (Lot-No. HPA09-0)

Mix No.	Allelic specificity	Size of the Specific Amplicon (basepairs)	Size of the Amplification Control (basepairs)	Mix No.	Allelic specificity	Size of the Specific Amplicon (basepairs)	Size of the Amplification Control (basepairs)
Mix No. 1a	HPA-1a	190	440	Mix No. 5a	HPA-5a	250	440
Mix No. 1b	HPA-1b	190	440	Mix No. 5b	HPA-5b	250	440
Mix No. 2a	HPA-2a	240	440	Mix No. 6a	HPA-6a	160	440
Mix No. 2b	HPA-2b	240	440	Mix No. 6b	HPA-6b	160	440
Mix No. 3a	HPA-3a	290	440	Mix No. 15a	HPA-15a	290	440
Mix No. 3b	HPA-3b	290	440	Mix No. 15b	HPA-15b	290	440
Mix No. 4a	HPA-4a	160	440	Negative Control	-	-	-
Mix No. 4b	HPA-4b	160	440	-	-	-	-

Table 2. Characteristics of the Human Platelet Antigens (HPA) detectable by using the HPA CTS-PCR-SSP Minitray Kit

System	Antigen	Alternative names	Phenotype frequency*	Glycoprotein	Nucleotide change	Amino acid change
HPA-1	HPA-1a	Zw ^a , Pl ^{A1}	97.9%	GPIIIa	T ¹⁹⁶	Leucine ³³
	HPA-1b	Zw ^b , Pl ^{A2}	28.8%		C ¹⁹⁶	Proline ³³
HPA-2	HPA-2a	Ko ^b	>99.9%	GPIb	C ⁵²⁴	Threonine ¹⁴⁵
	HPA-2b	Ko ^a , Sib ^a	13.2%		T ⁵²⁴	Methionine ¹⁴⁵
HPA-3	HPA-3a	Bak ^a , Lek ^a	80.95%	GPIIb	T ²⁶²²	Isoleucine ⁸⁴³
	HPA-3b	Bak ^b	69.8%		G ²⁶²²	Serine ⁸⁴³
HPA-4	HPA-4a	Yuk ^b , Pen ^a	>99.9%	GPIIIa	G ⁵²⁶	Arginine ¹⁴³
	HPA-4b	Yuk ^a , Pen ^b	<0.1%		A ⁵²⁶	Glutamine ¹⁴³
HPA-5	HPA-5a	Br ^b , Zav ^b	99.0%	GPIa	G ¹⁶⁴⁸	Glutamic acid ⁵⁰⁵
	HPA-5b	Br ^a , Zav ^a , Hc ^a	19.7%		A ¹⁶⁴⁸	Lysine ⁵⁰⁵
HPA-6	HPA-6a	Ca ^a , Tu ^a	(>99%)	GPIIIa	G ¹⁵⁶⁴	Arginine ⁴⁸⁹
	HPA-6b		0.7%		A ¹⁵⁶⁴	Glutamine ⁴⁸⁹
HPA-15	HPA-15a	Gov ^b	80.5%	CD109	C ²¹⁰⁸	Serine ⁷⁰³
	HPA-15b	Gov ^a	60.2%		A ²¹⁰⁸	Tyrosine ⁷⁰³

* Phenotype frequencies in Caucasian populations; markedly different frequencies may be found in other populations

Data published by the Institute for Biological Standards and Controls, UK (September 6, 2002).

Short Working Instruction for HPA-Typing with the HPA CTS-PCR-SSP Typing Kit

1. Isolate DNA and dilute to a concentration of 150 ng/μl.
2. Set up pre-PCR mixture (volume per test):

“Mastermix” (PCR buffer)	46 μl
Taq polymerase	0.96 μl *
Aqua dest.	109.6 μl

*Taq amount may vary depending on brand and lot; validation before use is recommended.

Vortex.

3. Before adding DNA, pipette **10 μl of the pre-PCR mixture to mix 15** on minitray (= negative control). Then add **16.6 μl of DNA suspension** (150 ng/μl) to the pre-PCR mixture. Vortex.
4. Aliquot **10 μl of the pre-PCR mixture** into each of the remaining cavities (**mix 1-14**).
5. Centrifuge the minitray briefly to pellet down the reagents.
6. Close the minitray with 8-cap stripe well.
7. Thermal cycling using the following program:

Initial denaturation	:	94°C, 2 min
		followed by 10 cycles of
Denaturation	:	94°C, 15 sec
Annealing & Extension	:	65°C, 1 min
		followed by 20 cycles of
Denaturation	:	94°C, 15 sec
Annealing	:	61°C, 50 sec
Extension	:	72°C, 30 sec
		eventually,
Hold	:	4°C, 15 min

8. Agarose gel electrophoresis (2% agarose).
9. Result interpretation using manual or worksheet.

Laboratory: _____

Name: _____

Date of Birth: _____

DNA-No. : _____

Remarks: _____

Primer Mix- No.	Specificity HPA *	Amp (bp)
1	HPA 1 - 1a	190
2	HPA 1 - 1b	190
3	HPA 2 - 2a	240
4	HPA 2 - 2b	240
5	HPA 3 - 3a	290
6	HPA 3 - 3b	290
7	HPA 4 - 4a	160
8	HPA 4 - 4b	160
9	HPA 5 - 5a	250
10	HPA 5 - 5b	250
11	HPA 6 - 6a	160
12	HPA 6 - 6b	160
13	HPA 15 - 15a	290
14	HPA 15 - 15b	290
15	Negative Control	-

Result

HPA	
1	,
2	,
3	,
4	,
5	,
6	,
15	,

Date: _____

Technician : _____

Lab. Supervisor: _____