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CTS Collaborative Transplant Study

WORKING INSTRUCTION
CYTOKINE GENOTYPING
Cytokine CTS-PCR-SSP TRAY KIT

LOCUS- AND LOT-SPECIFIC MANUAL

To be applied to the following product:

Product No.	Description	
124	Cytokine CTS- PCR-SSP Tray Kit	CE

Introduction

- This manual contains information on the Cytokine CTS-PCR-SSP Tray Kit for Cytokine genotyping available from the Collaborative Transplant Study (CTS). It should be used together with the Main Manual (Manual No. 100A, "Working Instruction for the CTS-PCR-SSP Tray and Minitray Kits") you received along with the kit.
- Cytokines are soluble proteins or glycoproteins produced mostly but not exclusively by leukocytes. They act as chemical communicators between cells but not as effector molecules in their own right and are mostly secreted but can be also expressed on the cell surface. They mostly bind on specific receptors on the surface of target cells and are either growth or differentiation factors.
- Cytokines show in general a low degree of polymorphism, although for a series of them there have been reports on existing isoforms. In most of the cases, polymorphisms are restricted to the promoter region of the cytokine genes. Several authors describe that those polymorphisms have both, functional and clinical relevance. Mutations in cytokine gene promoter sequences may alter specific transcription factor recognition sites and consequently affect transcriptional activation and cytokine production. Levels of various cytokines have been found to influence graft outcome: The TNF- α high production genotype has been associated with both increased acute and chronic kidney allograft rejection rates. There are conflicting reports about the effect of IL-10 on graft survival. A certain IFN- γ genotype is related to acute kidney graft rejection and to the development of fibrosis in lung transplants. Finally, a TGF- β 1 genotype is associated with fibrosis and chronic rejection of lung transplants.
- **Intended Use:** The Cytokine CTS-PCR-SSP Tray Kit which has been developed in our laboratory was used as the official reagent set for the Cytokine component of the 13th International Histocompatibility Workshop. It enables the user to detect some of the polymorphisms described in the promoter regions of the IL-1 α , IL-1 β , IL-1R, IL-1RA, IL-2, IL-4, IL-6, IL-10, IL-12, TNF- α , and γ -IFN genes, as well as some polymorphisms in the translated regions of the TGF- β , and the IL-4R α genes. For all the above-mentioned polymorphisms, there have been reports that they have a functional relevance and that they are associated with high or low production of the corresponding cytokine. The method is a PCR-SSP assay, which in most cases allows the definition not only of the polymorphic variants themselves, but also of the haplotypes that are present in the individual you are testing.

Very important:
 Since January 2006, the tray/minitray/bulk layout has changed!
 Please pay special attention to the new mix positions on the tray/minitray/bulk rack!
 (see Main Manual)

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1. Kit Composition

Each tray of the Cytokine CTS-PCR-SSP Tray Kit contains PCR primer mixes prepipetted and lyophilized in thin-walled, plastic, green 96-well PCR trays for Cytokine genotyping of **two** individuals (**48 PCR primer mixes** for **each** individual). Each kit provides **10 trays** for **20 typings** in total.

1.1. Names and positions of mixes

The PCR mixes have been named numerically for each cytokine (i.e. IL-1 α Mix No. 1 and IL-1 α Mix No. 2; IL-1 β Mix No. 1 to IL-1 β Mix No. 4, etc.).

Please refer to Figure 1 and Table 1 for mix positions on tray.

1.2. Specificities of mixes

Please refer to Table 1 for further information on the specificity (detectable polymorphism) of each primer mix.

2. PCR reaction/PCR reaction buffer (Mastermix)

Perform DNA isolation, preparation of the PCR reaction mix, amplification in a thermal cycler and gel electrophoresis according to the instructions of the Main Manual (Manual No. 100A, 'Working Instruction for the CTS-PCR-SSP Tray and Minitray Kits').

Please note: Use the **Mastermix CYT** (provided along with the trays) which contains 5% glycerol as PCR reaction buffer (without Taq Polymerase).

3. Result Evaluation

3.1. Internal positive control (amplification control)

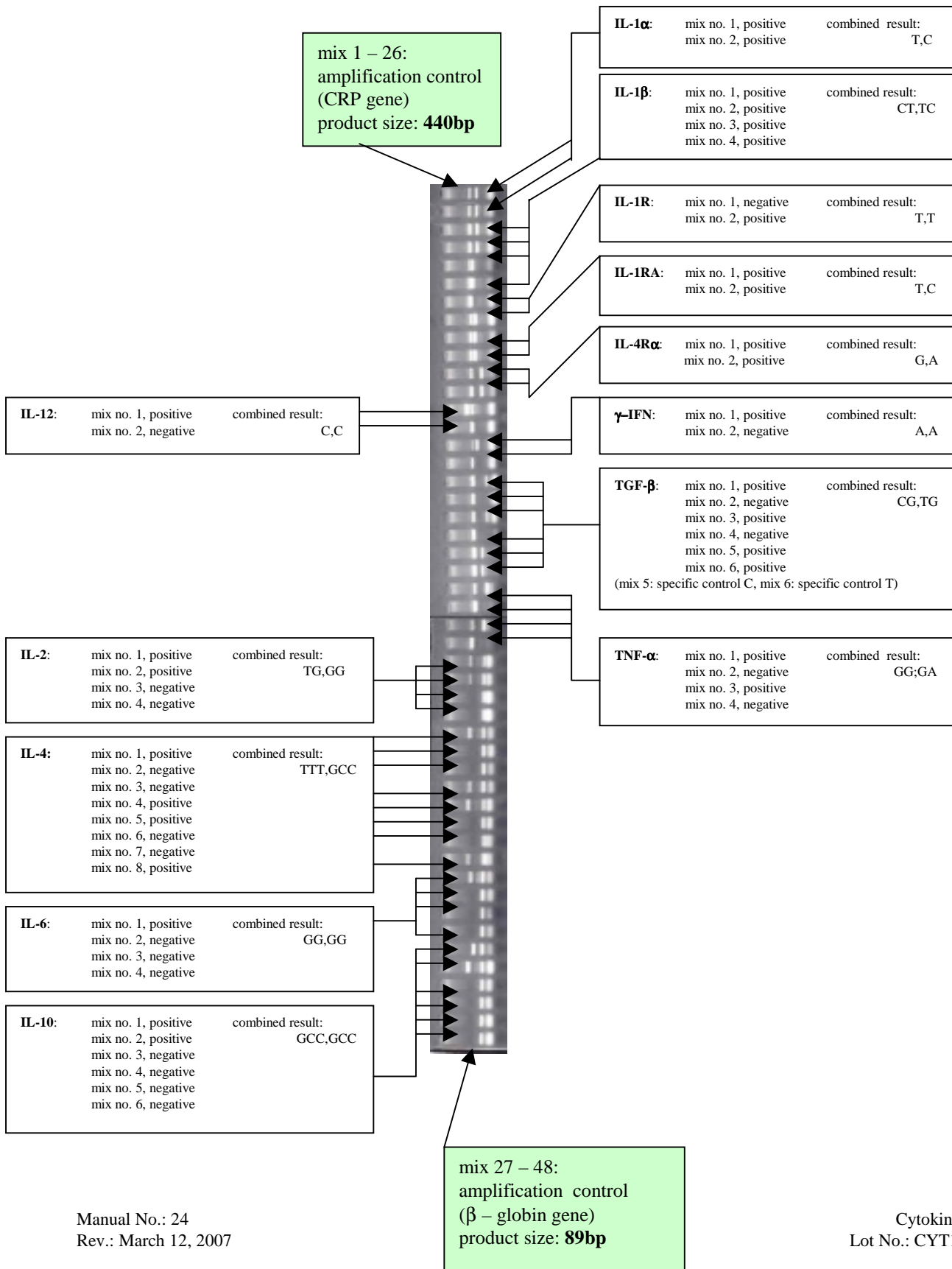
As internal positive control, a fragment of either the human C-reactive protein gene (89 bp) or the human β -globin gene (440 bp) will be amplified depending on the cytokine gene which is analyzed:

- The internal positive control primer pairs included in the PCR-SSP primer mix for typing of the following cytokines amplify a **440 bp** fragment of the human C-reactive protein gene: IL-1 α , IL-1 β , IL-1R, IL-1Ra, IL-4R α , IL-12, γ I-FN, TGF- β , TNF- α .
- The internal positive control primer pairs included in the PCR-SSP primer mix for typing of the following cytokines amplify a **89 bp** fragment of the human β -globin gene: IL-2, IL-4, IL-6, IL-10.

3.2. Allele-specific amplification products

Slots in which only an allele-specific PCR product is present (and often no or only a weak internal positive control band) indicate the presence of the allele-specific sequence (polymorphism) of the cytokine gene analyzed.

3.3. Example



4. Interpretation Hints

Some of the cytokine reagents allow detection of certain haplotype motives which give characteristic amplification patterns, consisting of at least one positive reaction. It is very important to distinguish between PCR mixes which give rise to a short (β -globin) or a long (CRP) amplification control band (internal positive control) (see section 3.1. above)!

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.

Special notes

- Mix positions D6+D12 (IL-10, mix no. 3) and mix B6+B12 (IL-10, mix no. 5) sometimes produce faint nonspecific bands, depending on the DNA concentration and purity.
- Mix H3+H9 (TGF- β , mix no. 1) and mix G3+G9 (TGF- β , mix no. 2) show tendency towards primer dimer formation which should not be mistaken for an allele-specific band. The allele-specific fragment is, if positive, 80 bp long and shows a strong signal.

If you have any particular questions concerning this kit which are not answered in this or in the Main Manual, please do not hesitate to contact me or my co-workers at:

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Figure 1. Position on Tray

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

(black marker line)

Starting position: H1, followed by G1, F1, etc.

Table 1: Position, PCR fragment size and allele specificities of each PCR-SSP primer mix of the Cytokine CTS-PCR-SSP Tray Kit (Lot No.: CYT15)

Position on tray (see Figure 1)		Mix	Allelic specificity	Corresponding genotype/haplotype	Size of the allele-specific amplicon	Size of the amplifi- cation control
H1	H7	α	T at pos -889	T		
G1	G7	IL-1 α Mix No. 2	C at pos -889	C	220 bp	440 bp
F1	F7	IL-1 β Mix No. 1	C at pos -511	C	215 bp	440 bp
E1	E7	IL-1 β Mix No. 2	T at pos -511	T	215 bp	440 bp
D1	D7	IL-1 β Mix No. 3	T at pos +3962	T	336 bp	440 bp
C1	C7	IL-1 β Mix No. 4	C at pos +3962	C	336 bp	440 bp
B1	B7	IL-1R Mix No. 1	C at pos <i>ps1</i> 1970	C	288 bp	440 bp
A1	A7	IL-1R Mix No. 2	T at pos <i>ps1</i> 1970	T	288 bp	440 bp
H2	H8	IL-1RA Mix No.1	T at pos <i>mspa1</i> 11100	T	297 bp	440 bp
G2	G8	IL-1RA Mix No. 2	C at pos <i>mspa1</i> 11100	C	297 bp	440 bp
F2	F8	IL-4R α Mix No. 1	G at pos +1902	G	143 bp	440 bp
E2	E8	IL-4R α Mix No. 2	A at pos +1902	A	143 bp	440 bp
D2	D8	IL-12 Mix No. 1	C at pos -1188	C	802 bp	440 bp
C2	C8	IL-12 Mix No. 2	A at pos -1188	A	802 bp	440 bp
B2	B8	γ -IFN Mix No. 1	A at pos +874	A	277 bp	440 bp
A2	A8	γ -IFN Mix No. 2	T at pos +874	T	277 bp	440 bp
H3	H9	TGF- β Mix No. 1	C at Codon 10 ;G at Codon 25	CG	80 bp	440 bp
G3	G9	TGF- β Mix No. 2	C at Codon 10 ;C at Codon 25	CC	80 bp	440 bp
F3	F9	TGF- β Mix No. 3	T at Codon 10 ;G at Codon 25	TG	80 bp	440 bp
E3	E9	TGF- β Mix No. 4	T at Codon 10 ;C at Codon 25	TC	80 bp	440 bp
D3	D9	TGF- β Mix No. 5	C at Codon 10	CG or CC	195 bp	440 bp
C3	C9	TGF- β Mix No. 6	T at Codon 10	TG or TC	195 bp	440 bp
B3	B9	TNF- α Mix No. 1	G at pos -308 ;G at pos -238	GG	110 bp	440 bp
A3	A9	TNF- α Mix No. 2	A at pos -308 ;G at pos -238	AG	110 bp	440 bp
H4	H10	TNF- α Mix No. 3	G at pos -308 ;A at pos -238	GA	110 bp	440 bp
G4	G10	TNF- α Mix No. 4	A at pos -308 ;A at pos -238	AA	110 bp	440 bp
F4	F10	IL-2 Mix No. 1	T at pos -330; G at pos +166	TG	562 bp	89 bp
E4	E10	IL-2 Mix No. 2	G at pos -330, G at pos +166	GG	564 bp	89 bp
D4	D10	IL-2 Mix No. 3	G at pos -330; T at pos +166	GT	569 bp	89 bp
C4	C10	IL-2 Mix No. 4	T at pos -330; T at pos +166	TT	569 bp	89 bp
B4	B10	IL-4 Mix No. 1	T at pos-1098 ;T at pos -590	TT*	557 bp	89 bp
A4	A10	IL-4 Mix No. 2	T at pos-1098 ;C at pos-590	TC*	557 bp	89 bp
H5	H11	IL-4 Mix No. 3	G at pos-1098 ;T at pos-590	GT*	557 bp	89 bp
G5	G11	IL-4 Mix No. 4	G at pos-1098 ;C at pos-590	GC*	557 bp	89 bp
F5	F11	IL-4 Mix No. 5	T at pos-590 ;T at pos-33	*TT	610 bp	89 bp
E5	E11	IL-4 Mix No. 6	T at pos-590 ;C at pos-33	*TC	610 bp	89 bp
D5	D11	IL-4 Mix No. 7	C at pos-590 ;T at pos-33	*CT	610 bp	89 bp
C5	C11	IL-4 Mix No. 8	C at pos-590 ;C at pos-33	*CC	610 bp	89 bp
B5	B11	IL-6 Mix No. 1	G at pos -174; G at pos <i>nt565</i>	GG	427 bp	89 bp
A5	A11	IL-6 Mix No. 2	C at pos -174; G at pos <i>nt565</i>	CG	426 bp	89 bp
H6	H12	IL-6 Mix No. 3	G at pos -174; A at pos <i>nt565</i>	GA	428 bp	89 bp
G6	G12	IL-6 Mix No. 4	C at pos -174; A at pos <i>nt565</i>	CA	428 bp	89 bp
F6	F12	IL-10 Mix No. 1	G at pos -1082; C at pos-819	GC* GCC or GCA	305 bp	89 bp
E6	E12	IL-10 Mix No. 2	G at pos -1082; C at pos-592	G*C GCC or GAC	530 bp	89 bp
D6	D12	IL-10 Mix No. 3	A at pos -1082; C at pos-819	AC* ACC or ACA	305 bp	89 bp
C6	C12	IL-10 Mix No. 4	A at pos -1082; T at pos-819	AT* ATC or ATA	305 bp	89 bp
B6	B12	IL-10 Mix No. 5	A at pos -1082; C at pos-592	A*C ACC or ATC	530 bp	89 bp
A6	A12	IL-10 Mix No. 6	A at pos -1082; A at pos-592	A*A ACAor ATA	530 bp	89 bp