

M University Clinic Heidelberg
Department of Transplantation Immunology
Im Neuenheimer Feld 305
D-69120 Heidelberg - Germany
Phone: +49-6221-564013
Fax: +49-6221-564200
www.ctstransplant.org


Manual No.: 24
Revision: November 28, 2011
Product No.: 124
Lot No.: CYT20

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	

Changes compared with lot CYT19:

- There are no changes in the number of mixes or mix specificities.

Introduction

- **Intended Use:** This manual contains information on the Cytokine CTS-PCR-SSP Tray Kit available from the Collaborative Transplant Study (CTS) for genotyping of cytokine polymorphisms. 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.
- 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 polymorphisms mentioned above, 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.
- 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 allograft outcome after transplantation.

Table of contents

Section	Description	Page
1.	Kit Composition	3
2.	PCR reaction/Mastermix	3
3.	Result Evaluation	3
4.	Interpretation Hints	5
Appendix	Figure 1	6
	Table 1	7

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) as PCR reaction buffer. DNA Polymerase is not included in the kit.

3. Result Evaluation

3.1. Internal positive control (amplification control)

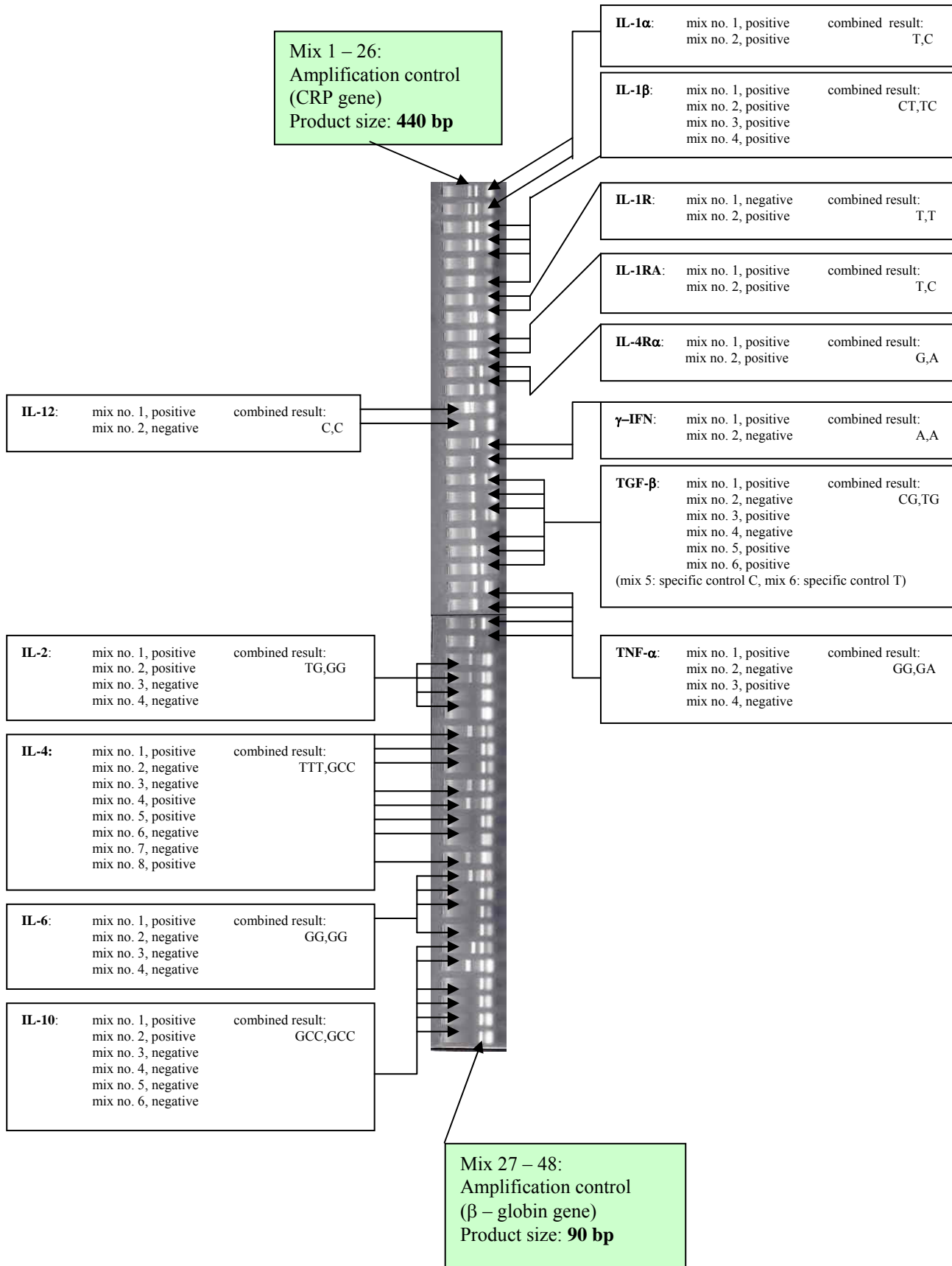
As internal positive control, a fragment of either the human β -globin gene (440 bp) or the human C-reactive protein gene (90 bp) will be amplified depending on the cytokine gene which is analyzed:

- 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 , and TNF- α .
- The internal positive control primer pairs included in the PCR-SSP primer mix for typing of the following cytokines amplify a **90 bp** fragment of the human β -globin gene: IL-2, IL-4, IL-6, and 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 crucial factors. If your bands are too weak or if you obtain non-specific bands, you might try to adjust these two factors until you obtain optimal results.

Special notes

- **γ -IFN:** The allele-specific band of mix 1, if visible, may appear weaker than the allele-specific band of mix 2 and should not be interpreted as non-specific reaction.
- **TGF- β :** Mix 1 and 2 may cause non-specific smear. This should be distinguished from the allele-specific PCR fragments which are relatively small (80 base pairs).
- **IL-4:** Mix 6 may produce faint non-specific amplification. An allele-specific product is strongly positive.

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:

Tel.: ++49 6221 564013

Fax: ++49 6221 564200

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

Hien Tran, M.D.

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, E1 etc. The next row starts at position H2 and so on.

Table 1: Mix position, allele specificities and PCR fragment size of each PCR-SSP primer mix of the Cytokine CTS-PCR-SSP Tray Kit (Lot No. **CYT20**)

Position on tray (Figure 1)		Mix name	Allelic specificity (based on SNP nomenclature commonly used in literature)	Corresponding genotype/haplotype§	Size of the allele-specific amplicon (base pairs)	Size of the amplifi- cation control (bp)	Cytokine Official Full Name†	Cytokine Official Symbol†	dbSNP-ID†
H1	H7	IL-1 α Mix No. 1	<i>T at pos -889</i>	T	220 bp	440 bp	<i>Interleukin 1, alpha</i>	<i>IL1A</i>	<i>rs1800587</i>
G1	G7	IL-1 α Mix No. 2	<i>C at pos -889</i>	C	220 bp	440 bp			
F1	F7	IL-1 β Mix No. 1	<i>C at pos -511</i>	C	215 bp	440 bp	<i>Interleukin 1, beta</i>	<i>IL1B</i>	<i>C/T at -511: rs16944</i>
E1	E7	IL-1 β Mix No. 2	<i>T at pos -511</i>	T	215 bp	440 bp			
D1	D7	IL-1 β Mix No. 3	<i>T at pos +3962</i>	T	340 bp	440 bp			<i>T/C at +3962: rs1143634</i>
C1	C7	IL-1 β Mix No. 4	<i>C at pos +3962</i>	C	340 bp	440 bp			
B1	B7	IL-1R Mix No. 1	<i>C at pos pst1 1970</i>	C	290 bp	440 bp	<i>Interleukin 1 receptor type 1</i>	<i>IL1R1</i>	<i>rs2234650</i>
A1	A7	IL-1R Mix No. 2	<i>T at pos pst1 1970</i>	T	290 bp	440 bp			
H2	H8	IL-1RA Mix No.1	<i>T at pos mspa1 11100</i>	T	300 bp	440 bp	<i>Interleukin 1 receptor antagonist</i>	<i>IL1RN</i>	<i>rs315952</i>
G2	G8	IL-1RA Mix No. 2	<i>C at pos mspa1 11100</i>	C	300 bp	440 bp			

Position on tray (Figure 1)		Mix name	Allelic specificity (based on SNP nomenclature commonly used in literature)	Corresponding genotype/haplotype§	Size of the allele-specific amplicon (base pairs)	Size of the amplifi- cation control (bp)	Cytokine Official Full Name†	Cytokine Official Symbol†	dbSNP-ID†
F2	F8	IL-4R α Mix No. 1	<i>G at pos +1902</i>	G	140 bp	440 bp	<i>Interleukin 4 receptor</i>	<i>IL4R</i>	<i>rs1801275</i>
E2	E8	IL-4R α Mix No. 2	<i>A at pos +1902</i>	A	140 bp	440 bp			
D2	D8	IL-12 Mix No. 1	<i>C at pos -1188</i>	C	800 bp	440 bp	<i>Interleukin 12B</i>	<i>IL12B</i>	<i>rs3212227</i>
C2	C8	IL-12 Mix No. 2	<i>A at pos -1188</i>	A	800 bp	440 bp			
B2	B8	γ -IFN Mix No. 1	<i>A at pos +874</i>	A	180 bp	440 bp	<i>Interferon, gamma</i>	<i>IFNG</i>	<i>rs2430561</i>
A2	A8	γ -IFN Mix No. 2	<i>T at pos +874</i>	T	180 bp	440 bp			
H3	H9	TGF- β Mix No. 1	<i>C at Codon 10 ; G at Codon 25</i>	CG	80 bp	440 bp	<i>Transforming growth factor, beta 1</i>	<i>TGFB1</i>	<i>SNP in codon 10: rs1800470</i>
G3	G9	TGF- β Mix No. 2	<i>C at Codon 10 ; C at Codon 25</i>	CC	80 bp	440 bp			
F3	F9	TGF- β Mix No. 3	<i>T at Codon 10 ; G at Codon 25</i>	TG	80 bp	440 bp			
E3	E9	TGF- β Mix No. 4	<i>T at Codon 10 ; C at Codon 25</i>	TC	80 bp	440 bp			
D3	D9	TGF- β Mix No. 5	<i>C at Codon 10</i>	CG or CC	195 bp	440 bp			<i>SNP in codon 10: rs1800470</i>
C3	C9	TGF- β Mix No. 6	<i>T at Codon 10</i>	TG or TC	195 bp	440 bp			

Position on tray (Figure 1)		Mix name	Allelic specificity (based on SNP nomenclature commonly used in literature)	Corresponding genotype/haplotype§	Size of the allele-specific amplicon (base pairs)	Size of the amplifi- cation control (bp)	Cytokine Official Full Name†	Cytokine Official Symbol†	dbSNP-ID†
B3	B9	TNF- α Mix No. 1	<i>G at pos -308 ; G at pos -238</i>	GG	110 bp	440 bp	<i>Tumor necrosis factor (TNF superfamily, member 2)</i>	<i>TNF</i>	<i>G/A at -308: rs1800629 G/A at -238: rs361525</i>
A3	A9	TNF- α Mix No. 2	<i>A at pos -308 ; G at pos -238</i>	AG	110 bp	440 bp			
H4	H10	TNF- α Mix No. 3	<i>G at pos -308 ; A at pos -238</i>	GA	110 bp	440 bp			
G4	G10	TNF- α Mix No. 4	<i>A at pos -308 ; A at pos -238</i>	AA	110 bp	440 bp			
F4	F10	IL-2 Mix No. 1	<i>T at pos -330 ; G at pos +166</i>	TG	560 bp	90 bp	<i>Interleukin 2</i>	<i>IL2</i>	<i>T/G at -330: rs2069762 G/T at +166: rs2069763</i>
E4	E10	IL-2 Mix No. 2	<i>G at pos -330 ; G at pos +166</i>	GG	560 bp	90 bp			
D4	D10	IL-2 Mix No. 3	<i>G at pos -330 ; T at pos +166</i>	GT	570 bp	90 bp			
C4	C10	IL-2 Mix No. 4	<i>T at pos -330 ; T at pos +166</i>	TT	570 bp	90 bp			

Position on tray (Figure 1)		Mix name	Allelic specificity (based on SNP nomenclature commonly used in literature)	Corresponding genotype/haplotype§	Size of the allele-specific amplicon (base pairs)	Size of the amplifi- cation control (bp)	Cytokine Official Full Name†	Cytokine Official Symbol‡	dbSNP-ID†	
B4	B10	IL-4 Mix No. 1	<i>T at pos-1098 ; T at pos -590</i>	TT*	560 bp	90 bp	Interleukin 4	IL4	<i>T/G at -1098: rs2243248</i>	
A4	A10	IL-4 Mix No. 2	<i>T at pos-1098 ; C at pos -590</i>	TC*	560 bp	90 bp				
H5	H11	IL-4 Mix No. 3	<i>G at pos-1098 ; T at pos -590</i>	GT*	560 bp	90 bp				
G5	G11	IL-4 Mix No. 4	<i>G at pos-1098 ; C at pos -590</i>	GC*	560 bp	90 bp				
F5	F11	IL-4 Mix No. 5	<i>T at pos-590 ; T at pos -33</i>	*TT	610 bp	90 bp			<i>T/C at -590: rs2243250</i>	
E5	E11	IL-4 Mix No. 6	<i>T at pos-590 ; C at pos -33</i>	*TC	610 bp	90 bp				
D5	D11	IL-4 Mix No. 7	<i>C at pos-590 ; T at pos -33</i>	*CT	610 bp	90 bp				<i>T/C at -33: rs2070874</i>
C5	C11	IL-4 Mix No. 8	<i>C at pos-590 ; C at pos -33</i>	*CC	610 bp	90 bp				
B5	B11	IL-6 Mix No. 1	<i>G at pos -174 ; G at pos +565</i>	GG	430 bp	90 bp	Interleukin 6 (interferon, beta 2)	IL6	<i>G/C at -174: rs1800795</i> <i>G/A at +565: rs1800797</i>	
A5	A11	IL-6 Mix No. 2	<i>C at pos -174 ; G at pos +565</i>	CG	430 bp	90 bp				
H6	H12	IL-6 Mix No. 3	<i>G at pos -174 ; A at pos +565</i>	GA	430 bp	90 bp				
G6	G12	IL-6 Mix No. 4	<i>C at pos -174 ; A at pos +565</i>	CA	430 bp	90 bp				

Position on tray (Figure 1)		Mix name	Allelic specificity (based on SNP nomenclature commonly used in literature)	Corresponding genotype/haplotype§	Size of the allele-specific amplicon (base pairs)	Size of the amplifi- cation control (bp)	Cytokine Official Full Name†	Cytokine Official Symbol†	dbSNP-ID†
F6	F12	IL-10 Mix No. 1	<i>G at pos -1082 ; C at pos -819</i>	GC* = GCC or GCA	305 bp	90 bp	<i>Interleukin 10</i>	<i>IL10</i>	<i>G/A at -1082: rs1800896</i> <i>C/T at -819: rs1800871</i> <i>C/A at -592: rs1800872</i>
E6	E12	IL-10 Mix No. 2	<i>G at pos -1082 ; C at pos -592</i>	G*C = GCC or GTC	530 bp	90 bp			
D6	D12	IL-10 Mix No. 3	<i>A at pos -1082 ; C at pos -819</i>	AC* = ACC or ACA	305 bp	90 bp			
C6	C12	IL-10 Mix No. 4	<i>A at pos -1082 ; T at pos -819</i>	AT* = ATC or ATA	305 bp	90 bp			
B6	B12	IL-10 Mix No. 5	<i>A at pos -1082 ; C at pos -592</i>	A*C = ACC or ATC	530 bp	90 bp			
A6	A12	IL-10 Mix No. 6	<i>A at pos -1082 ; A at pos -592</i>	A*A = ACA or ATA	530 bp	90 bp			

§ Haplotypes of TGF-β and IL-10: „ bold“ haplotypes are those which are frequently found in Caucasoids.

† „Cytokine Official Full Name“, „Cytokine Official Symbol“ and “dbSNP identification” (rs number) are based on www.ncbi.nlm.gov (“Gene” database and “SNP” database, respectively).

Please note the different sizes of the amplification controls (small fragment for IL-2, IL-4, IL-6 and IL-10, shaded).