

PRODUCT INSERT

CYTOKINE GENOTYPING TRAY

For Research Use Only. Not for use in diagnostic procedures.

Catalog Number	Description	Cytokines	Number of Tests	Number of D-Mix Tubes	Tray Seals	Number of PCR Reactions per Test	D-mix Volume μl per Test	DNA Volume μl per Test	Taq Polymerase Volume μl per Test
CYTGEN	Cytokine Genotyping Tray	TGF- β 1, TNF- α , IL-6, IL-10, IFN- γ	24	24	6	16	180	19	1

INTENDED USE

A test assay for genotyping of the following cytokine factors:

- Transforming growth factor - β 1 (TGF- β 1)
- Tumor necrosis factor - α (TNF- α)
- Interleukin - 6 (IL-6)
- Interleukin - 10 (IL-10)
- Interferon - γ (IFN- γ)

These four factors have been selected based on published investigative interest in their possible roles in transplant rejection.

SUMMARY AND EXPLANATION

Cytokines are crucial to cellular communication and, thus, may be key elements in turning on and off the immune response. Studies have linked the genotypes TNF- α , TGF- β 1, IL-6, and IL-10 with the expression levels of these cytokines. Furthermore, these cytokine genotypes may be relevant to an organ transplant patient's immunological response to the graft. This cytokine genotyping tray will identify those genotypes whose expression levels have been observed to be associated with transplant rejection.

PRINCIPLE(S)

The PCR-SSP methodology is based on the principle that completely matched oligonucleotide primers are more efficiently used in amplifying a target sequence than a mismatched oligonucleotide primer by recombinant Taq polymerase. Primer pairs are designed to have perfect matches only with a single allele or group of alleles. Under strictly controlled PCR conditions, perfectly matched primer pairs result in the amplification of target sequences (i.e., a positive result), while mismatched primer pairs do not result in amplification (i.e., a negative result).

After the PCR process, the amplified DNA fragments are separated by agarose gel electrophoresis and are visualized by staining with ethidium bromide and exposure to ultraviolet light. Interpretation of PCR-SSP results is based on the presence or absence of a specific amplified DNA fragment. Since amplification during the PCR reaction may be adversely affected by various factors (pipetting errors, poor DNA quality, presence of inhibitors, etc.) an internal control primer pair is included in every PCR reaction. The control primer pair amplifies a conserved region of the Human β -globin gene, which is present in all DNA samples and is used to verify the integrity of the PCR reaction. In the presence of a positive typing band (specific amplification of a cytokine allele), the product of the internal control primer may be weak or absent due to the difference in concentration and melting temperatures between the specific primer pairs and the internal control primer pair. The amplified DNA fragments of the specific cytokine primer pairs are smaller than the product of the internal control primer pair, but larger than the diffuse, unincorporated primer band. Thus, a positive reaction for a specific cytokine allele or allele group is visualized on the gel as an amplified DNA fragment between the internal control product band and the unincorporated primer band.

REAGENTS

A. Identification

The Cytokine Genotyping Tray provides sequence-specific oligonucleotide primers for amplification of selected TNF- α , TGF- β 1, IFN- γ , IL-6, and IL-10 alleles and the human β -globin gene by the polymerase chain reaction (PCR). These alleles are known to be associated with the expression level of these factors.¹⁻¹⁰ Pre-optimized primers are presented (dried) in different wells of a 96-well 0.2 ml thin-walled tube tray for PCR and are ready for the addition of DNA samples, recombinant Taq polymerase, and specially formulated dNTP-buffer mix (D-mix). Each tray includes a negative control reaction tube that detects the presence of the internal control product generated by the tray. The internal control PCR product, amplified from the human β -globin gene is the most likely contaminating PCR product due to its amplification in every well. The amount of primer is adjusted for optimal

amplification of 100 ng of sample DNA when used in conjunction with the D-mix, the prescribed amount of recombinant Taq polymerase, and the PCR reaction profile detailed below. See the provided worksheet for specific alleles which can be amplified by each primer set under the specified PCR conditions of the assay. For lot specific primer site locations, please refer to the worksheet.

B. Warning or Caution

Note: There are no specific safety concerns for materials supplied by One Lambda. Other warnings pertain to materials not provided by One Lambda. In all cases the manufacturer's MSDS should be consulted for specific warnings and guidelines.

1. For Research Use Only. Not for use in diagnostic procedures.
2. Carcinogen Warning: The ethidium bromide used for staining of DNA is a potential carcinogen. Always wear gloves when handling stained gels.
3. Biohazard Warning: All blood products should be treated as potentially infectious.
4. Caution: Wear UV-blocking eye protection, and do not view UV light source directly when viewing or photographing gels.
5. Pipettes used for **Post**-PCR manipulations should **not** be used for **Pre**-PCR manipulations.

C. Instructions for Use

See "Directions For Use."

D. Storage Instructions

Store reagents at temperature indicated on package. Use before printed expiration date.

E. Purification or Treatment Required for Use

1. Any primer sets with cracks in the tubes or on the lip, which may affect complete sealing of the reaction tubes to prevent evaporative losses, should be considered unusable.
2. If salts have precipitated out of the solution in the D-mix aliquots during shipping or storage, re-dissolve by extended vortexing at room temperature (20 - 25°C).
3. D-mix aliquots, upon thawing at room temperature (20 - 25°C), should be pink to light purple in color. Any D-mix aliquot without the specified coloration should be considered unusable.

F. Instability Indications

1. Any primer sets with cracks in the tubes or on the lip, which may affect complete sealing of the reaction tubes to prevent evaporative losses, should be considered unusable.
2. If salts have precipitated out of the solution in the D-mix aliquots during shipping or storage, re-dissolve by extended vortexing at room temperature (20 - 25°C).
3. D-mix aliquots, upon thawing at room temperature (20 - 25°C), should be pink to light purple in color. Any D-mix aliquot without the specified coloration should be considered unusable.

INSTRUMENT REQUIREMENTS

A. Programming the Perkin Elmer 9600 or 9700 Thermocycler.

The following program is designed to program 96-Well GeneAmp® PCR System 9600, 9700 or Veriti™ 96-Well Thermal Cycler (Applied Biosystems). Set ramp speed to 9600 for 96-Well GeneAmp® PCR System 9700. Set ramp speed to 9600 Emulation Mode for Veriti™ 96-Well Thermal Cycler. Set reaction volume to 10 µl volume. Program the thermal cycler before starting the "Step-by-Step procedure" below.

One Lambda PCR Program (OLI-1)

# of Cycles	Step	Temp. (°C)	Time (sec.)
1	1	96	130
	2	63	60
9	1	96	10
	2	63	60
20	1	96	10
	2	59	50
	3	72	30
End	1	4	---

For specific thermal cycler information, refer to the manufacturer's user manual.

B. 2.5% Agarose Gel Preparation (for Gel System, OLI Cat. #MGS108)

- a. To set up:
 - Slide the locking pin on the base to the open position.
 - Insert the gel box into the base matching the color-coded sides to assure proper orientation.
 - Lock the gel box into the base by sliding the locking pin into the locked position.
 - Use the leveling bubble and the three height adjustable legs to level the base.
- b. Orient and fully insert the 14 gel combs into the gel comb holder.
- c. To 100 ml of 1x Tris Borate EDTA buffer (1xTBE) with 0.5µg/ml ethidium bromide (in a 500 ml glass bottle), add 2.5 g electrophoresis grade agarose. Heat until a homogeneous solution is formed.
- d. Add 30 ml of the gel solution to the gel box. Make sure the agarose covers the entire surface evenly by tilting the gel box back and forth immediately after the gel solution is added. Quickly place the gel comb holder on the filled gel box by matching the color coding. Allow to set for 15 minutes.
- e. Remove the gel combs by lifting the gel comb holder while holding the base. Add 10 ml of 1x TBE containing 0.5 µg/ml ethidium bromide evenly across the gel to fill every well.

C. Gel Electrophoresis (for Gel System, OLI Cat. #MGS108)

- a. After completing the PCR Reaction:
 - Orient the DNA primer set tray and gel box with the negative control well in the upper left hand corner.
 - Gently remove the tray seal without splashing the samples.
- b. Transfer each PCR reaction (10µl) in sequence to the 2.5% agarose gel. Make sure to transfer all samples in the proper sequence. (No addition of electrophoresis dye is necessary.) Use of an 8- or 12- channel Pipetman[®] is recommended.
Note: The order of samples (to match the worksheet) is from left to right, top to bottom.
- c. Cover the gel box with the gel box cover by matching the color-coded sides. Electrophorese the samples at 140 - 150 volts until the red tracking dye has migrated about 0.5 cm into the gel (approximately 3 - 5 minutes, depending on the agarose used). Remove the cover.
- d. Slide the locking pin on the base to the open position and remove the gel box. Transfer the gel box to a UV transilluminator. Photograph the completed gel.
- e. Orient the photograph with the negative control reaction in the upper left corner, and mark the corresponding positive allele groups on the worksheet provided with the tray.

SPECIMEN COLLECTION AND PREPARATION

1. DNA can be purified from human leukocytes by any preferred method.
2. The DNA sample to be used for PCR-SSP analysis should be re-suspended in sterile water or in 10 mM Tris-HCl, pH 8.0 - 9.0 at a concentration of 25 - 200 ng/µl with the A260/A280 ratio of 1.65 - 1.80.
3. **Samples should not be re-suspended in solutions containing chelating agents, such as EDTA, above 0.5 mM in concentration.**
4. DNA samples may be used immediately after isolation or stored at -20°C or below for extended periods of time (over 1 year) with no adverse effects on results.
5. DNA samples should be shipped at 4°C or below to preserve their integrity during transport.

PROCEDURE

A. Materials Provided

- Four 96-well microtiter[®] trays with dried primers in each reaction well
- 24 tubes D-mix (180 µl each)
- 6 tray seals

B. Materials Required, But Not Provided

- Pipetting devices, such as: Gilson[®] P-20, Gilson[®] P200 Pipetman[®]
- Disposable pipette tips
- Vortex mixer
- Microcentrifuge
- PCR tray microtube storage rack and cover (Robbins Scientific, Cat. #1044-39-5)
Pressure pad (OLI Cat. #SSPPAD) *Note: The pressure pad is good for a maximum of 300 PCR runs. Please order a new pad if the surface of the pad that contacts the tray seal is no longer smooth.*
- for use with tray seal

- 96-Well GeneAmp® PCR System 9600, 9700 or Veriti™ 96-Well Thermal Cycler (Applied Biosystems). Set ramp speed to 9600 for 96-Well GeneAmp® PCR System 9700. Set ramp speed to 9600 Emulation Mode for Veriti™ 96-Well Thermal Cycler.
- Hot plate or microwave oven for heating agarose solutions
- Electrophoresis apparatus/power supply (150V minimum capacity)
- UV transilluminator (Example: Fotodyne FOTO/UV®21)
- Photographic or image documentation system

Recombinant Taq polymerase (Perkin-Elmer AmpliTaq® DNA Polymerase—5 units/μl)

- C. Step-by-step procedure.
See “Directions For Use.”

DIRECTIONS FOR USE

A. Sample Preparation

1. Purify genomic DNA from leukocyte sample by method of choice. Final DNA concentration should be 25 - 200ng/μl (100ng/μl is optimal) with the A260/A280 ratio between 1.65-1.80.
2. For specific information on sample preparation and storage, see **Specimen Collection and Preparation** above.
3. Perform PCR on the purified DNA sample using Cytokine Genotyping Tray, or store DNA sample at -20°C or below until ready to type.

B. Reagent/Equipment Preparation

4. Program a thermal cycler to run the One Lambda PCR program. (See “Instrument Requirements” above.)
5. Have available: recombinant Taq polymerase (5 units/μl). Store at -20°C.
6. Prepare electrophoresis gel (2.5% agarose) using the Gel System (OLI Cat. #MGS108) or with at least 96 sample wells (space rows of wells at least 1 cm apart).

- C. **Instructions for Pipetting Taq Polymerase:** To avoid waste, please follow the simple instructions listed below for pipetting Taq polymerase.

Note: Taq polymerase is very viscous and special care must be taken in the aliquoting process. Failure to follow the steps described below may result in reagent loss.

1. Pipette slowly, using a calibrated Gilson® Pipetman®. (A P10 Pipetman® is recommended for increased accuracy.)
2. The pipette tip should just pass the surface layer of the liquid.
Warning: Do not immerse the pipette tip in the liquid.
3. Carefully wipe excess liquid from pipette tip on the rim of the vial.

D. Stepwise Procedure

1. Remove from the indicated storage temperature: the volume (tube) of the D-Mix for the Cytokine Genotyping primer set tray, the primer set tray(s), and the appropriate number of DNA samples. Thaw at room temperature (20 - 25°C).

Note: You may cut tray into the number of tests needed for a single work session. Immediately return the un-used portion of tray to the appropriate storage temperature.

•Vortex DNA samples to mix.

•Place the primer set tray in a PCR Tray Microtube Storage Rack (Robbins Scientific, Cat. #1044-39-5) and remove the tray label.










2. Remove recombinant Taq polymerase from -20°C, and keep on ice until ready to use.
3. Using a Pipetman® (or equivalent), add 1 μl of DNA diluent to the negative control reaction tube on the primer set tray.
- E. Using a Pipetman®, add recombinant Taq polymerase (5 units/μl) to the D-mix tube. (See chart at the beginning of this document for amount.)
4. Cap tube and vortex for 5 seconds. Pulse-spin the D-mix tube in a microcentrifuge to bring all liquid down from sides of the tube.
5. Using a P20 Pipetman®, pipette 9 μl of the D-mix to the negative control reaction tube.
6. Using a Pipetman®, add the DNA sample to the D-mix tube. (See chart at the beginning of this document for amount.)
7. Cap tube and vortex for 5 seconds. Pulse-spin the D-mix tube in a microcentrifuge.
8. Using a P20 Pipetman® or an electronic Pipetman®, aliquot 10 μl of the sample-reaction mixture from the D-mix tube into each reaction tube, **except the negative control reaction tube**, of the primer set tray.
Important: Be sure to apply the sample above the primers (dried at the bottom of each reaction tube) to avoid cross-contamination between tubes. Touch the inside wall of the tube with the pipette tip to allow the sample to slide down to the bottom of the tube. Check that all samples have dropped to the bottom of each tube. If not, tap the tray gently on the bench top so that all samples settle at the bottom of the tube before you begin PCR.
9. Cover the reaction tubes with the tray seal provided. Check that all reaction tubes are completely covered by the tray seal to prevent evaporative loss during PCR.
10. Place the primer set tray in the thermal cycler.
11. Place a pressure pad on top of the tray before closing the thermal cycler.

LIMITATIONS OF THE PROCEDURE

1. PCR-SSP is a dynamic process requiring highly controlled conditions to ensure discriminatory amplification. The procedures provided in this product must be strictly followed.
2. The extracted sample DNA provides the template for the specific amplification process, and, thus, must have its concentration and purity within the ranges specified in the procedure.
3. All instruments (e.g., PCR thermal cycler, pipetting devices) must be calibrated according to the manufacturer's recommendations.
4. This assay does not diagnose any clinical condition nor level of risk. It detects only genotypes of selected cytokine genes.

EXPECTED VALUES

Gel Interpretation*

	Positive Reaction	Negative Reaction	Non-amplification
Well			
Internal Control Band			
Positive Typing Band			
Primer Band			

*The internal control band and broad unincorporated primer band serve as size markers. Any visible band between the two size markers should be considered positive typing bands.

SPECIFIC PERFORMANCE CHARACTERISTICS

Not applicable; product for research use only.

BIBLIOGRAPHY

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REVISION HISTORY

Revision	Date	Revision Description
4	2005/02	Clarify intended use; update references and template.
5	2009/02	Added the Veriti™ Thermal Cycler in the Instrument Requirements Section; Separated “Thermal Cycler” into two words.
6	2009/12	A typographical error was corrected in the Summary and Explanation Section; a redundant statement was also removed from the Summary and Explanation Section.