MILLIPLEX™ MAP

TGFβ1,2,3 MILLIPLEX KIT
96 Well Plate Assay

#TGFB-64K-03

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FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

The transforming growth factor beta (TGFβ) system, a superfamily of cytokines as well as signaling pathways, is highly conserved throughout the animal kingdom. TGFβ functions in angiogenesis, wound healing and embryonic development, and plays a critical role in immunity, heart disease, and cancer. In its normal state TGFβ is one of the few classes of proteins able to inhibit cell growth by halting mitosis at the G1 state, inducing cell differentiation or apoptosis. However, during oncogenesis mutations in the TGFβ signaling pathway result in tumor cell resistance to the effects of normally functioning TGFβ, causing proliferation without regulation. Initial research suggests that VE-cahedrin may enhance the mutated TGFβ signaling pathway, while other research indicates that DNA methylation plays a role in pathway mutation.

The secreted TGFβ cytokine exists in three isoforms: TGFβ1, TGFβ2 and TGFβ3. Secreted by most immune cells, TGFβ1 plays a critical role in controlling the immune system, acting on cells differently depending on cell type as well as stage of differentiation. TGFβ2, also known as glioblastoma-derived T-cell suppressor factor (G-TSF), plays a role in embryonic development and has the ability to suppress the effects of interleukin-dependent T-cell tumors. TGFβ3 regulates cellular adhesion molecules and extracellular matrix formation, as well as lung and palate development. TGFβ3 deficiency during mammalian development results in the cleft palate deformity. In addition TGFβ3 controls wound healing by regulating epidermal and dermal cell movement in injured skin.

TGFβ3 may not be detected in normal plasma and serum samples. Due to the involvement of all three isotypes in neonate development and lactation regulation, literature suggests detectable levels in milk.

This kit is for research purposes only.

Please read entire protocol before use.

It is important to use same assay incubation conditions throughout your study.

PRINCIPLE

MILLIPLEX™ MAP is based on the Luminex® xMAP® technology — one of the fastest growing and most respected multiplex technologies offering applications throughout the life sciences and capable of performing a variety of bioassays including immunoassays on the surface of fluorescent-coded beads known as microspheres.

- Luminex® uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, 100 distinctly colored bead sets can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin-PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- The microspheres are allowed to pass rapidly through a laser which excites the internal dyes marking the microsphere set. A second laser excites PE, the fluorescent dye on the reporter molecule.
- Finally, high-speed digital-signal processors identify each individual microsphere and quantify the result of its bioassay based on fluorescent reporter signals.

The capability of adding multiple conjugated beads to each sample results in the ability to obtain multiple results from each sample. Open-architecture xMAP® technology enables multiplexing of many types of bioassays reducing time, labor and costs over traditional methods.

STORAGE CONDITIONS UPON RECEIPT

- Recommended storage for kit components is 2 - 8°C.
- Once the standards and controls have been reconstituted, immediately transfer contents into polypropylene vials. **DO NOT STORE RECONSTITUTED STANDARDS OR CONTROLS IN GLASS VIALS**. For long-term storage, freeze reconstituted standards and controls at ≤ -20°C. Avoid multiple (>2) freeze/thaw cycles.
- **DO NOT FREEZE Antibody-Immobilized Beads, Detection Antibodies, and Streptavidin-Phycoerythrin**.

REAGENTS SUPPLIED

Note: Store all reagents at 2 – 8°C

REAGENTS SUPPLIED	CATALOG NUMBER	VOLUME	QUANTITY
TGFβ1,2,3 Standard	TGFB-8064-3	lyophilized	1 vial
TGFβ1,2,3 Quality Controls 1 and 2	TGFB-6064-3	lyophilized	2 vials
TGFβ Detection Antibody	LTGF-1064	3.2 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE9	3.2 mL	1 bottle
Serum Matrix Note: Contains 0.08% Sodium Azide	LMC-SD	lyophilized	1 vial (required for serum and plasma samples only)
Sample Diluent Note: Contains 0.08% Sodium Azide	LTGF-SD	5.0 mL	1 bottle
1.0 N Hydrochloric Acid (HCl)	L-HCL	1.0 mL	1 bottle
1.0 N Sodium Hydroxide (NaOH)	L-NAOH	1.0 mL	1 bottle
Assay Buffer	L-MAB1	30 mL	2 bottles
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	1 bottle
Set of one 96-Well Filter Plate with 2 Sealers	MX-PLATE	----------	1 plate 2 sealers

Bead/Analyte Name	Luminex Bead Region	(1X concentration, 3.5 mL) Available	Cat. #
Anti-TGFβ1,2,3 Premixed Beads	19,41,79	✓	TGFB-PMX3

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Luminex Sheath Fluid (Luminex Catalogue #40-50000)

Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 5 µL to 1000 µL
2. Multichannel Pipettes capable of delivering 5 µL to 50 µL or 25 µL to 200 µL
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Rubber Bands
6. Absorbent Pads
7. Laboratory Vortex Mixer
8. Sonicator (Branson Ultrasonic Cleaner Model #B200 or equivalent)
9. Titer Plate Shaker (Lab-Line Instruments Model #4625 or equivalent)
10. Vacuum Filtration Unit (Millipore Vacuum Manifold Catalog #MSVMHTS00 or equivalent with Millipore Vacuum Pump Catalog #WP6111560 or equivalent)
11. Luminex 100™ IS, 200™, or HTS by Luminex Corporation
12. Plate Stand (Millipore Catalog # MX-STAND)

SAFETY PRECAUTIONS

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

TECHNICAL GUIDELINES

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.

- The bottom of the Microtiter Filter Plate should not be in direct contact with any surface during assay set-up or incubation times. The plate can be set on a plate stand or on the non-flat side of the plate cover or any other plate holder to raise the plate from the surface. A plate stand can be purchased separately from Millipore (Millipore Catalog #MX-STAND).
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- After the wash steps, keep the bottom of the Microtiter Filter Plate clean by blotting on paper towels or absorbent pads to prevent any leakage due to capillary action.
- Keep the vacuum suction on the plate as low as possible. It is recommended to have a vacuum setting that will remove 200 µL of buffer in $\geq$ 5 seconds (equivalent to < 100 mmHg).
- After hydration, all Standards and Controls must be transferred to polypropylene tubes.
- The Standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be stored at ≤ -20°C for 1 month and at ≤ -80°C for greater than one month.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- Any unused mixed Antibody-Immobilized Beads may be stored in the Mixing Bottle at 2-8°C for up to one month.
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some analytes.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7 which is approximately 500-800 rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes. Adjust probe height according to the protocols recommended by Luminex to the kit filter plate using 3 alignment discs prior to reading an assay.
- For cell culture supernatants or tissue extraction, use the culture or extraction medium as the matrix solution in background, standard curve and control wells. If samples are diluted in Assay Buffer, use the Assay Buffer as matrix.
- For serum/plasma samples, use the Serum Matrix provided in the kit.
- For cell/tissue homogenate, the final cell or tissue homogenate should be prepared in a buffer that has a neutral pH, contains minimal detergents or strong denaturing detergents, and has an ionic strength close to physiological concentration. Avoid debris, lipids, and cell/tissue aggregates. Centrifuge samples before use.
- Vortex all reagents well before adding to plate.

SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- See Preparation of Reagents Step E for detailed sample treatment information before setting up the assay.

B. Preparation of Plasma Samples:

- Centrifuge at 10,000xg for 10 min at 2 - 8° C within 30 minutes of collection for complete platelet removal. Remove plasma and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- See Preparation of Reagents Step E for detailed sample treatment information before setting up the assay.

C. Preparation of Tissue Culture Supernatant:

- Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- Tissue culture supernatant may require a dilution with an appropriate control medium prior to assay.
- See Preparation of Reagents Step E for detailed sample treatment information before setting up the assay.

NOTE:

- A maximum of 25 µL per well of tissue culture medium or diluted serum/plasma samples can be used.
- To measure circulating TGFβ1 in plasma it is necessary to remove platelets from the samples. Platelet granules contain TGFβ1 which is released upon activation.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**

PREPARATION OF REAGENTS FOR IMMUNOASSAY

A. Preparation of Antibody-Immobilized Beads

Sonicate the antibody-bead vial for 30 seconds; vortex for 1 minute. Unused portion may be stored at 2-8°C for up to six months.

B. Preparation of Quality Controls

Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 µL **Assay Buffer**. Invert the vial several times to mix and vortex. Allow the vial to sit for 5-10 minutes and then transfer the controls to appropriately labeled polypropylene microfuge tubes. Unused portion may be stored at ≤ -20°C for up to one month.

C. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water. Store unused portion at 2-8°C for up to one month.

D. Preparation of Serum Matrix

This step is required for serum/plasma samples only.

Add 1.0 mL of deionized water and 4.0 mL of Assay Buffer to the bottle containing the lyophilized Serum Matrix, mix and let it set for at least 10 min at room temperature to allow complete reconstitution. Mix well. In a separate tube, add 0.5 mL of the reconstituted Serum Matrix to 0.5 mL Assay Buffer for a **final 1:10 dilution**. Mix well. This is the working Serum Matrix to be used for the Standards and Controls when measuring serum /plasma samples.

E. Treatment of Samples

For measuring active TGFβ, it is necessary to treat serum/plasma samples or serum-containing tissue/cell culture samples prior to the assay. Serum-free samples do not require further treatment. The following sample treatment protocols are recommended for serum/plasma and serum-containing samples. **Note: Serum-containing tissue/cell culture samples (e.g. fetal bovine serum-containing DMEM) may or may not require dilution, depending on the sample concentration of TGFβ.**

Serum/Plasma Samples:

1. Centrifuge samples to remove debris and excess lipids.
2. Dilute serum/plasma samples by adding 1 part of serum/plasma to 4 parts of Sample Diluent (e.g. 20 µl serum and 80 µl Cat. # LTGF-SD).
3. Add 2.0 µl of 1.0 N HCl to each 25 µl of the 1:5 diluted samples. Make sure sample pH drops below 3.0. After mixing, acidified samples should be moderately shaken at room temperature for 15 min or incubated with no shaking for 1 hour at room temperature.
4. Acid-treated serum/plasma samples should be further diluted 1:6 using Assay Buffer (Cat. # L-MAB) as the diluent for a **final sample dilution of 1:30** immediately prior to addition to sample wells.

Serum Containing Tissue/Cell Culture Samples Requiring Dilution:

1. Centrifuge samples to remove debris and excess lipids.
2. Dilute serum-containing tissue/cell culture samples with Sample Diluent. The dilution factor should be determined by the user. For example, adding 1 part of sample to 4 parts of Sample Diluent (e.g. 20 µl sample and 80 µl Cat. # LTGF-SD) for 1:5 dilution.
3. Add 1.0 µl of 1.0 N HCl to each 25 µl of the 1:5 diluted serum-containing culture medium samples. Make sure sample pH drops below 3.0. After mixing, acidified samples should be moderately shaken at room temperature for 15 min or incubated with no shaking for 1 hour at room temperature.
4. The acid-treated serum-containing tissue/cell culture samples should be neutralized by 1.0N NaOH prior to addition to sample wells. The volume of 1.0 N NaOH required is approximately similar to the volume of 1.0 N HCl used. But actual volume of 1.0 N NaOH required to achieve neutral pH may vary, depending on initial sample pH and buffering capacity of the samples.

Serum Containing Tissue/Cell Culture Samples Requiring **NO DILUTION**:

1. Centrifuge samples to remove debris and excess lipids.
2. Add 1.0 µl of 1.0 N HCl to each 25 µl of the serum-containing culture medium samples. Make sure sample pH drops below 3.0. After mixing, acidified samples should be moderately shaken at room temperature for 15 min or incubated with no shaking for 1 hour at room temperature.
3. The acid-treated serum-containing tissue/cell culture samples should be neutralized by 1.0N NaOH prior to addition to sample wells. The volume of 1.0 N NaOH required is approximately similar to the volume of 1.0 N HCl used. But actual volume of 1.0 N NaOH required to achieve neutral pH may vary, depending on initial sample pH and buffering capacity of the samples.

E. Preparation of TGFβ1,2,3 Standard

1.) Before use, reconstitute the TGFβ1,2,3 Standard with 250 µL **Assay Buffer** to give a 10,000 pg/mL concentration of standard. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow vial to sit 5-10 minutes for complete reconstitution. Transfer the standard to an appropriately-labeled polypropylene microfuge tube. This will be used as the 10,000 pg/mL standard; the unused portions may be stored at ≤ -20°C for up to one month.

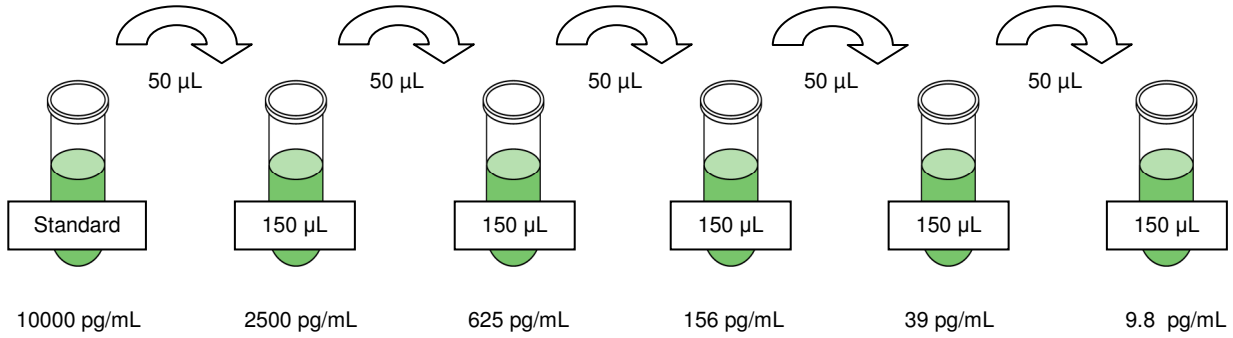
2.) Preparation of Working Standards

Label five polypropylene microfuge tubes 2500, 625, 156, 39,and 9.8 pg/mL. Add 150 µL of the Assay Buffer to each of the five tubes. Prepare serial dilutions by adding 50 µL of the 10,000 pg/mL reconstituted standard to the 2500 pg/mL tube, mix well and transfer 50 µL of the 2500 pg/mL standard to the 625 pg/mL tube, mix well and transfer 50 µL of the 625 pg/mL standard to the 156 pg/mL tube, mix well and transfer 50 µL of the 156 pg/mL standard to 39 pg/mL tube, mix well and transfer 50 µL of the 39 pg/mL standard to the 9.8 pg/mL tube and mix well. The 0 pg/mL standard (Background) will be the Assay Buffer.

Preparation of Working Standards

Standard Concentration (pg/mL)	Volume of **Assay Buffer** to Add	Volume of Standard to Add
10000	250 µL	0

Standard Concentration (pg/mL)	Volume of Assay Buffer to Add	Volume of Standard to Add
2500	150 µL	50 µL of 10000 pg/mL
625	150 µL	50 µL of 2500 pg/mL
156	150 µL	50 µL of 625 pg/mL
39	150 µL	50 µL of 156 pg/mL
9.8	150 µL	50 µL of 39 pg/mL



IMMUNOASSAY PROCEDURE

- Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.
- Allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Diagram the placement of Standards [0 (Background), 9.8, 39, 156, 625, 2500, 10000 pg/mL], Controls 1 and 2, and Samples on Well Map Worksheet in a vertical configuration. (Note: Most instruments will only read the 96-well plate vertically by default.) It is recommended to run the assay in duplicate.
- Set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.

1. Prewet the filter plate by pipetting 200 µL of Assay Buffer into each well of the Microtiter Filter Plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).
2. Remove Assay Buffer by vacuum. **(NOTE: DO NOT INVERT PLATE.)** Blot excess Assay Buffer from the bottom of the plate with an absorbent pad or paper towels.
3. Add 25 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for the 0 ng/mL standard (Background).
4. Add 25 µL of Assay Buffer to the sample wells.
5. Add 25 µL of appropriate matrix to the background, standards, and control wells. When assaying tissue culture samples, use identical control medium (provided by users) for Background, Standard and Quality Control wells. For serum/plasma samples, use the Serum Matrix (see Preparation of Reagents Step D.) for Background, Standard, and Quality Control wells.
6. Add 25 µL of treated sample into the appropriate wells. (See Preparation of Reagents Section Step E.)
7. Vortex Mixing Bottle and add 25 µL of the Mixed Beads to each well. (Note: During addition of Beads, shake bead bottle intermittently to avoid settling.)
8. Seal the plate with a plate sealer and cover it with the lid. Wrap a rubber band around the plate holder, plate and lid and incubate with agitation on a plate shaker for 2 hours at room temperature (20-25°C) or overnight (16-18 hours) at 4°C.

Add 200 µL Assay Buffer per well

↓ Shake 10 min, RT

↓ Vacuum

- Add 25 µL Standard or Control to appropriate wells
- Add 25 µL Assay Buffer to background and sample wells
- Add 25 µL appropriate matrix to Background, Standards and Control wells
- Add 25 µL treated samples to sample wells
- Add 25 µL Beads to each

↓ Incubate 2 hours at RT or overnight at 4°C with shaking

9. Gently remove fluid by vacuum. **(NOTE: DO NOT INVERT PLATE.)**
10. Wash plate 2 times with 200 µL/well of Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Blot excess Wash Buffer from the bottom of the plate with an absorbent pad or paper towels.
11. Add 25 µL of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)
12. Seal, cover with lid, and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C). **DO NOT VACUUM AFTER INCUBATION.**
13. Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.
14. Seal, cover with lid and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
15. Gently remove all contents by vacuum. **(NOTE: DO NOT INVERT PLATE.)**
16. Wash plate 2 times with 200 µL/well Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Wipe any excess buffer on the bottom of the plate with a tissue.
17. Add 100 µL of Sheath Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes.
18. Run plate on Luminex 100™ IS, 200™, or HTS.
19. Save and analyze the Median Fluorescent Intensity (MFI) data using a weighted 5-parameter logistic or spline curve-fitting method for calculating TGFB1,2,3 concentrations in samples.

Vacuum and wash 2X with 200 µL Wash Buffer

Add 25 µL Detection Antibodies per well

Incubate 1 hour at RT

Do Not Vacuum

Add 25 µL Streptavidin-Phycoerythrin per well

Incubate for 30 minutes at RT

Vacuum and wash 2X with 200 µL Wash Buffer

Add 100 µL Sheath Fluid per well

Read on Luminex (50 µL, 50 beads per bead set)

EQUIPMENT SETTINGS

These specifications are for the Luminex 100™ IS v.1.7 or Luminex 100™ IS v2.1/2.2, Luminex 200™ v2.3, xPONENT, and Luminex HTS. Luminex instruments with other software (e.g. MasterPlex, StarStation, LiquiChip, Bio-Plex, LABScan100) would need to follow instrument instructions for gate settings and additional specifications from the vendors.

Events:	50, per bead	
Sample Size:	50 µL	
Gate Settings:	8,000 to 15,000	
Reporter Gain:	Default (low PMT)	
Time Out:	60 seconds	
Bead Set:	Premixed TGF β1,2,3	Bead Region
	TGFβ1	19
	TGFβ2	41
	TGFβ3	79

QUALITY CONTROLS

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert or can be located at the MILLIPORE website www.millipore.com/techlibrary/index.do using the catalog number as the keyword.

ASSAY CHARACTERISTICS

Assay Sensitivities (minimum detectable concentrations, ng/mL)

MinDC: Minimum Detectable Concentration is calculated by the StatLIA® Immunoassay Analysis Software from Brendan Technologies. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions.

Cytokine	MinDC (pg/ml)
TGFβ1	12
TGFβ2	6
TGFβ3	6

Precision

Intra-assay precision is generated from the mean of the %CV's from five reportable results across two different concentrations of cytokines in one experiment. Inter-assay precision is generated from the mean of the %CV's from five reportable results each for three different concentrations of cytokine across six different experiments.

Cytokine	Intra-Assay % CV	Inter-Assay % CV
TGFβ1	5	13
TGFβ2	5	10
TGFβ3	5	9

Accuracy

Spike Recovery: The data represent mean percent recovery of multiple levels of spiked standards in multiple independent experiments.

Cytokine	Average % Recovery
TGFβ1	83
TGFβ2	105
TGFβ3	46

Cross-Reactivity

1. Cross-reactivities for the TGFβ1 and TGFβ3 assays were not detectable. The TGFβ2 assay had cross-reactivity with TGFβ1 of less than 5% and with TGFβ3 of less than 3%.
2. Besides human TGFβ1, the assay can detect TGFβ1 of rat, mouse, cynomolgous monkey, horse, rabbit, dog, cat, guinea pig, pig and hamster, but the exact amount of cross reactivity has not been determined.
3. Besides human TGFβ2, the assay can detect TGFβ2 of rat, mouse, cynomolgous monkey, horse, rabbit, dog, guinea pig, pig and hamster, but the exact amount of cross reactivity has not been determined.

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Filter plate will not vacuum	Vacuum pressure is insufficient	Increase vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds.
	Samples have insoluble particles	Centrifuge samples just prior to assay set-up and use supernatant. If high lipid concentration, after centrifugation, remove lipid layer and use supernatant.
	Sample too viscous	May need to dilute sample.
Insufficient bead count	Vacuum pressure too high	Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds.
	Bead mix prepared incorrectly	Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting into the plate.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with alcohol flush, backflush and washes; or, if needed, probe should be removed and sonicated.
	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
Plate leaked	Vacuum pressure too high	Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (prewetted) plate and continue.
	Plate set directly on table or absorbent towels during incubations or reagent additions	Set plate on plate stand or raised edge so bottom of filter is not touching any surface.
	Insufficient blotting of filter plate bottom causing wicking	Blot the bottom of the filter plate well with absorbent towels after each wash step.
	Pipette touching plate filter during additions	Pipette to the side of well.
	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately and by pipeting with multichannel pipets without touching reagent in plate.
	Matrix used has endogenous analyte or interference	Check matrix ingredients for crossreacting components (e.g. interleukin modified tissue culture medium).
	Insufficient washes	Increase number of washes.

Beads not in region or gate	Luminex not calibrated correctly or recently	Calibrate Luminex based on instrument manufacturer's instructions at least once a week or if temperature has changed by >3°C.
	Gate settings not adjusted correctly	Some Luminex instruments (e.g. Bio-Plex) require different gate settings than those described in the kit protocol. Use instrument default settings.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex 4 times to eliminate air bubbles. Wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.
	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.
Signal for whole plate is same as background	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue.
	Streptavidin-Phycoerythrin was not added	Add Streptavidin-Phycoerythrin according to protocol. If Detection Antibody has already been vacuumed out, sensitivity may be low.
Low signal for standard curve	Detection Antibody may have been vacuumed out prior to adding Streptavidin Phycoerythrin	May need to repeat assay if desired sensitivity not achieved.
	Incubations done at incorrect temperatures, timings or agitation	Assay conditions need to be checked.
Signals too high, standard curves are saturated	Calibration target value set too high	With some Luminex instruments (e.g. Bio-Plex) default target setting for RP1 calibrator is set at High PMT. Use low target value for calibration and reanalyze plate.
	Plate incubation was too long with standard curve and samples	Use shorter incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte	If below detectable levels, it may be possible to use higher sample volume. Check with tech support for appropriate protocol modifications.
	Samples contain analyte concentrations higher than highest standard point	Samples may require dilution and reanalysis for that particular analyte.
	Standard curve was saturated at higher end of curve	See above.

High variation in samples and/or standards	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm all reagents are vacuumed out completely in all wash steps.
	Samples may have high particulate matter or other interfering substances	See above.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using a vertical plate shaker at a speed where beads are in constant motion without splashing.
	Cross-well contamination	Check when reusing plate sealer that no reagent has touched sealer. Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.

REPLACEMENT REAGENTS	**CATALOG #**
TGFβ1,2,3 Standard	TGFB-8064-3
TGFβ1,2,3 Quality Controls	TGFB-6064-3
TGFβ Detection Antibodies	LTGF-1064
Sample Diluent	LTGF-SD
Steptavidin-Phycoerythrin	L-SAPE9
1.0 N HCl	L-HCL
1.0 N NaOH	L-NAOH
Assay Buffer	L-MAB1
Wash Buffer	L-WB
Set of two 96-Well Filter Plates with Sealers	MX-PLATE

Antibody-Immobilized Pre-Mixed Beads

Cytokine	Bead #	Cat. #
TGFβ1,2,3	19, 41, 79	TGFB-PMX3

ORDERING INFORMATION

To place an order:

To assure the clarity of your custom TGFB1,2,3 kit order, please FAX the following information to our customer service department:

- Your name, telephone and/or fax number
- Customer account number
- Shipping and billing address
- Purchase order number
- Catalog number and description of product
- Quantity of kits

FAX: (636) 441-8050
Toll-Free US: (866) 441-8400
(636) 441-8400

Mail Orders: Millipore Corp.
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

To best serve our international customers in placing an order or obtaining additional information about MILLIPLEX™ MAP products, please contact your multiplex specialist or sales representative or email our European Customer Service at customerserviceEU@Millipore.com.

Conditions of Sale

All products are for research use only. They are not intended for use in clinical diagnosis or for administration to humans or animals. All products are intended for *in vitro* use only.

Material Safety Data Sheets (MSDS)

Material Safety Data Sheets for Millipore products may be ordered by fax or phone or through our website at www.millipore.com/techlibrary/index.do.

WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 pg/mL Standard (Background)	625 pg/mL Standard	QC 2 Control									
B	0 pg/mL Standard (Background)	625 pg/mL Standard	QC 2 Control									
C	9.8 pg/mL Standard	2500 pg/mL Standard	Sample 1									
D	9.8 pg/mL Standard	2500 pg/mL Standard	Sample 1									
E	39 pg/mL Standard	10000 pg/mL Standard	Sample 2									
F	39 pg/mL Standard	10000 pg/mL Standard	Sample 2									
G	156 pg/mL Standard	QC 1 Control	Etc.									
H	156 pg/mL Standard	QC 1 Control										