Monkey TGF-β1 Immunoassay

Catalog Number: EA800175

For the quantitative determination of Monkey TGF-β1 concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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TABLE OF CONTENTS

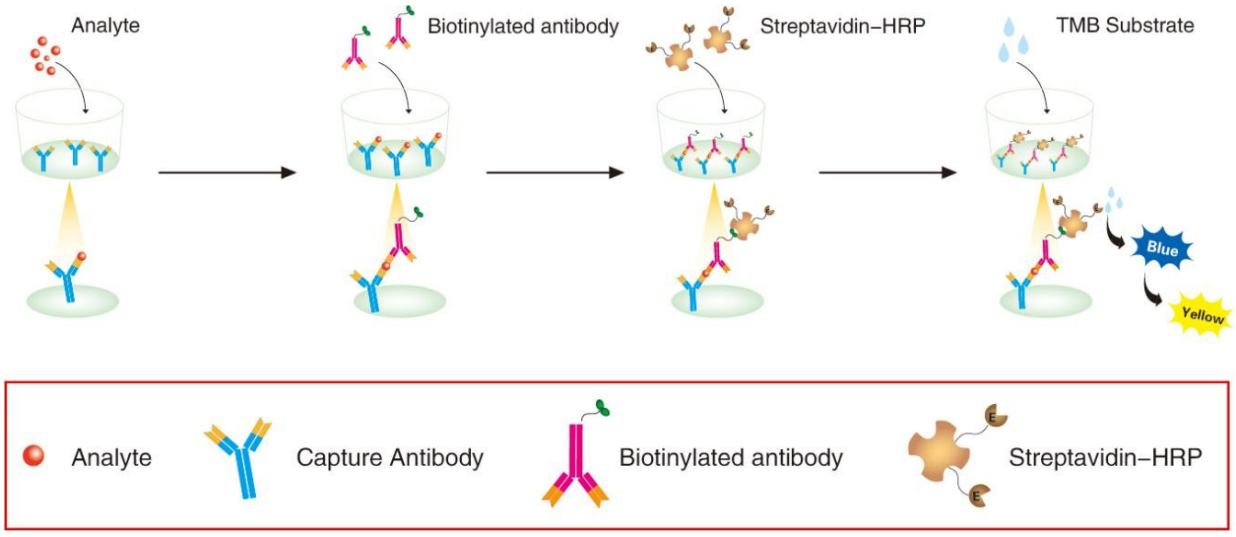
BACKGROUND

TGF-β1 (transforming growth factor beta 1) was first identified in human platelets as a protein with a molecular mass of 25 kilodaltons with a potential role in wound healing. TGF- β1, TGF- β2, and TGF- β3 all function through the same receptor signaling systems. They are members of the large TGF-β superfamily. TGF-β proteins are highly pleiotropic cytokines that regulate processes such as immune function, proliferation and epithelial mesenchymal transition. It was later characterized as a large protein precursor (containing 390 amino acids) that was proteolytically processed to produce a mature peptide of 112 amino acids .TGF-β activation from latency is controlled both spatially and temporally, by multiple pathways that include actions of proteases such as plasmin and MMP9, and/or by thrombospondin 1 or selected integrins. Although different isoforms of TGFβ are naturally associated with their own distinct LAPs, the TGF-β1 LAP is capable of complexing with, and inactivating, all other Monkey TGF-β isoforms and those of most other species. Mutations within the LAP are associated with Camurati Engelmann disease, a rare sclerosing bone dysplasia characterized by inappropriate presence of active TGF-β1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TGF-β1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for TGF-β1 is added to detect the captured TGF-β1 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



TECHNICAL HINTS AND LIMITATIONS

1. This ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

ORIGENE

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,2000pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
lyophilized Biotin-Conjugated antibody	1 vials	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP	1 vial	Store at 2-8°C** for six months
Standard /sample Diluent	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent	1 bottle	Store at 2-8°C** for six months
20 x Wash Buffer Concentrate	1 bottle	Store at 2-8°C** for six months
Substrate Solution	1 bottle	Store at 2-8°C** for six months
Stop Solution	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	
1N HCl	1 bottle	Store at 2-8°C** for six months
1N NaOH	1 bottle	Store at 2-8°C** for six months

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

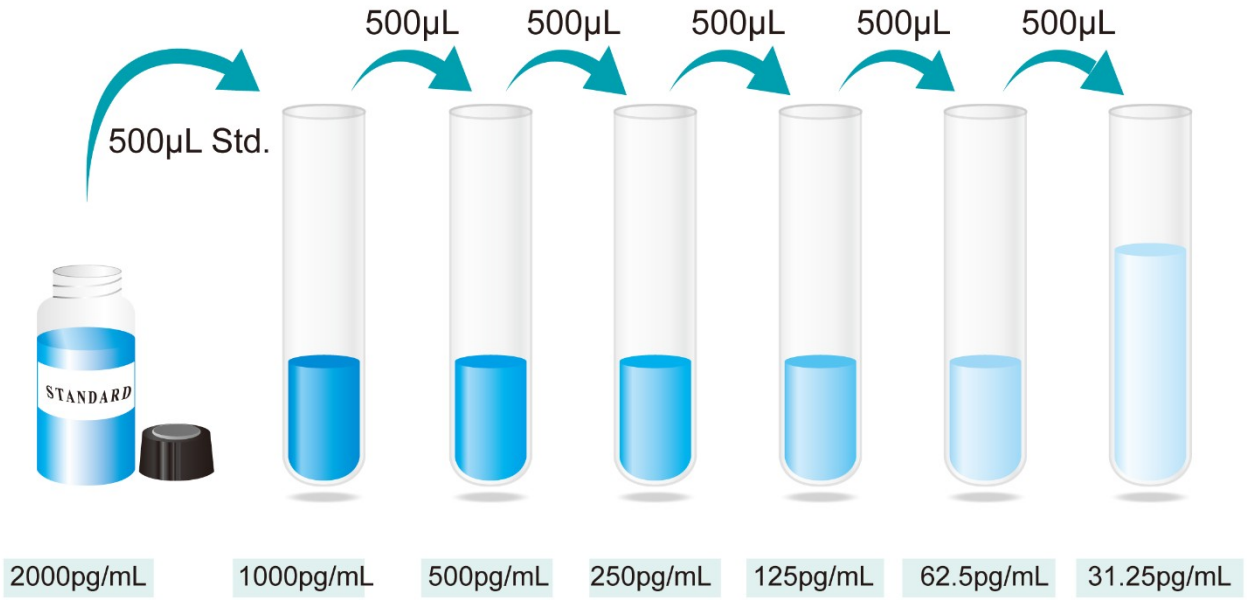
1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: The normal Monkey serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of 20x Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard\Sample (2 vials)** – Monkey TGF-β1 Standard has a total of 2 vials. Each vial contains the standard sufficient for generating a standard curve. Reconstitute the Standard with 1.0mL of deionized or distilled water. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500μL of Standard/Sample Diluent into 1000 pg/ml tube and the remaining tubes. Use the stock solution of 2000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly (vortex 20 sec for each of dilution step) and change pipette tips between each transfer. The 2000 pg/mL standard serves as the high standard. The Standard/sample Diluent serves as the zero standard (0 pg/mL).



Preparation of Monkey TGF-β1 standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-Monkey TGF-β1antibody(1 vials)** - The lyophilized Detection Antibody should be stored at 4℃ to -20℃ in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 110 µL of sterile Biotin-Conjugate antibody Diluent to each vial and vortex 30 sec to obtain the stock solution. If the entire 96-well plate is used, take 50µL of detection antibody stock solution into 10 mL of Biotin-Conjugate antibody Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial antibody is used. make a 1:200 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

5. **Working solution of Streptavidin-HRP(120µL)** - Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 120 µL HRP Conjugate sufficient for 96-well plate. Make 1:100 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 100 ul of HRP Conjugate to 10 mL of Streptavidin-HRP Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months. DO NOT FREEZE.

   ***The working solution should be used within one day after dilution.**

6. Prepare your samples before starting the test procedure as follows:

Activation of serum or plasma samples:

1) 5µl serum/plasma samples + 75µl Standard /sample Diluent

2) Add 10µl 1N HCl to 80µl pre-diluted sample, mix and incubate for 1hour at 2-8℃.

3) Neutralize by addition of 10µl 1N NaOH. The total volume is 100µl, and serum/plasma samples are 20-fold dilution.

4) Assay immediately or store samples at -20℃/-70℃ for 3 days. The results should be multiplied by the dilution factor.

***Note: TGF-β1 levels of different specimens may have great differences. Please dilute samples according to actual conditions.**

Activation of cell culture supernate samples:

1) 100µl cell culture supernates samples + 80µl Standard /sample Diluent

2) Add 10µl 1N HCl to 180µl pre-diluted sample, mix and incubate for 1hour at 2-8℃.

3) Neutralize by addition of 10µl 1N NaOH. The total volume is 200µl, and the samples are 2-fold dilution.

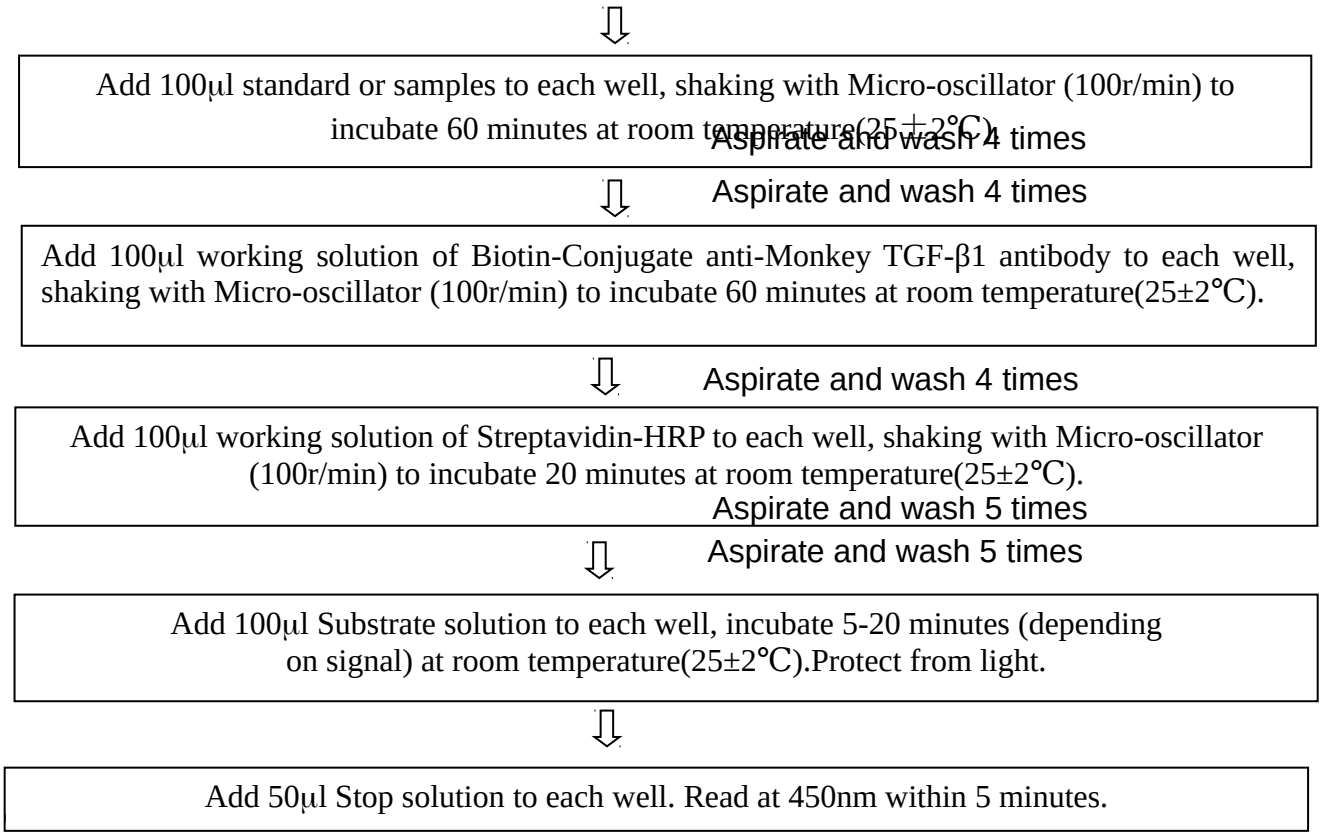
4) Assay immediately or store samples at -20℃/-70℃ for 3 days. The results should be multiplied by the dilution factor.

***Note: TGF-β1 levels of different specimens may have great differences. Please dilute samples according to actual conditions.**

***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

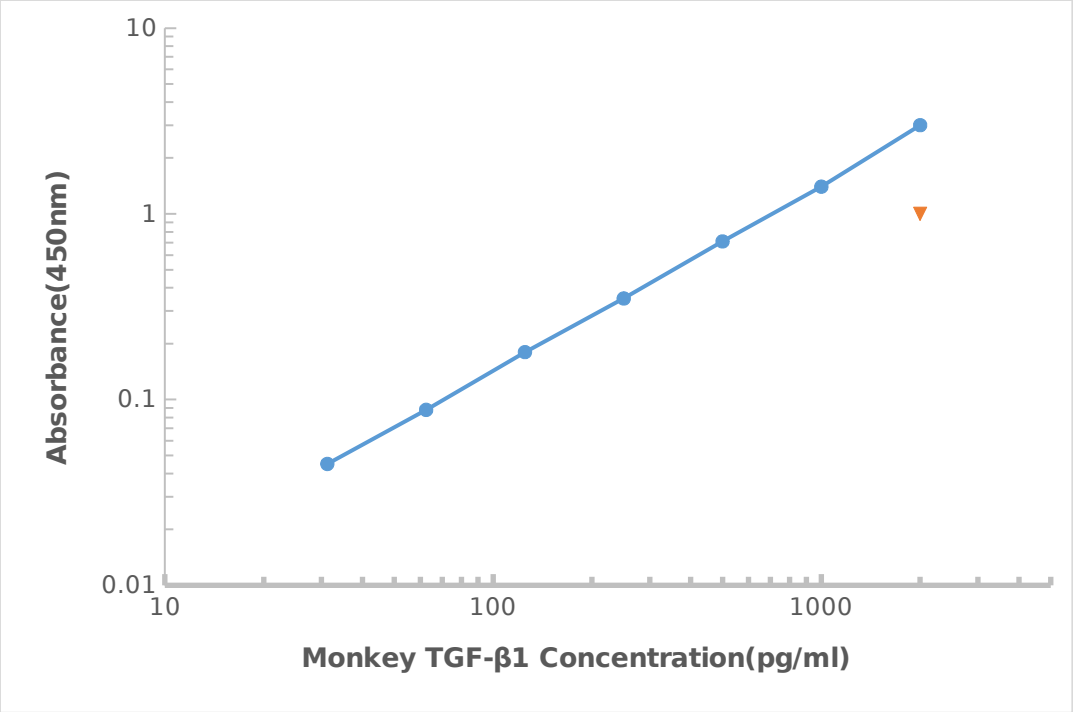


CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the TGF-B1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the TGF-β1 ELISA

Std (pg/mL)	O.D.1	O.D.2	Average	Corrected
0	**0.062**	**0.068**	**0.065**	**----**
31.25	**0.093**	**0.096**	**0.094**	**0.029**
62.5	**0.178**	**0.186**	**0.182**	**0.117**
125	**0.256**	**0.275**	**0.265**	**0.200**
250	**0.425**	**0.446**	**0.435**	**0.370**
500	**0.745**	**0.724**	**0.734**	**0.669**
1000	**1.358**	**1.339**	**1.348**	**1.283**
2000	**2.165**	**2.186**	**2.175**	**2.110**



Representative standard curve for TGF-β1 ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 6 pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant Monkey TGF-β1. The factors listed below were prepared at 10ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant Monkey	Recombinant mouse	Recombinant porcine
TGF-β2	TGF-beta1	
IL-2	IFN-gamma	
IL-4	IL-2	
IL-6		
IL-10		
TFN-α		
TGF-β3		
IFN-gamma		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of TGF-β1 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of TGF-β1 in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	92	84-101
Cell culture supernatants	95	87-104

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of TGF-β1 in various matrices and diluted with the appropriate Sample Diluent to

produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	95	102
	Range (%)	86-106	94-110
1:4	Average% of Expected	97	103
	Range (%)	89-105	96-113

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