

Human TGF-beta 1 ELISA Kit

Catalog No. CKH189

Quantity: 1 x 96 tests

Introduction

Transforming Growth Factor Beta (TGF- β) is a stable, multifunctional polypeptide growth factor. TGF- β exists in at least five isoforms, known as TGF- β 1, TGF- β 2, TGF- β 3, TGF- β 4, and TGF- β 5. Their amino acid sequences display homologies on the order of 70-80%. The various TGF- β isotypes share many biological activities and their actions on cells are qualitatively similar in most cases although there are a few examples of distinct activities. TGF- β 1 is the prevalent form and is found almost ubiquitously while the other isoforms are expressed in a more limited spectrum of cells and tissues. It is normally secreted as an inactive, or latent, complex.

Application

The Cell Sciences Human TGF- β 1 ELISA (Enzyme-Linked Immunosorbent Assay) Kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human TGF- β 1 in serum, plasma, cell culture supernatants and urine.

This assay employs an antibody specific for human TGF- β 1 coated on a 96-well plate. Standards and samples are pipetted into the wells and TGF- β 1 present in a sample is bound to the wells by the immobilizing antibody. The wells are washed and biotinylated anti-human TGF- β 1 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted into the wells. The wells are washed again, a TMB substrate solution is added to the wells, and color develops in the wells in proportion to the amount of TGF- β 1 bound. The Stop Solution changes the color of the wells from blue to yellow, and the intensity of the color is measured at 450 nm.

Performance and Characteristics:

Sensitivity

The minimum detectable dose of TGF- β 1 is typically less than 80 pg/ml.

Reproducibility

Intra-Assay: CV<10%

Inter-Assay: CV<12%



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Recovery

Recovery was determined by spiking various levels of Human TGF- β 1 into Human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	94.46	82-102
Plasma	95.78	93-103
Cell culture media	97.87	85-104

Linearity

Sample Type		Serum	Plasma	Cell culture media
1:2	Average % of Expected	92	95	95
	Range (%)	82-103	83-104	84-104
1:4	Average % of Expected	93	94	94
	Range (%)	83-105	84-105	83-104

Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., human ANG, CD23, Eotaxin, GCSF, GM-CSF, GRO- α , GRO- β , GRO- γ , I-309, IFN- γ , IL-1 α , IL-1 β , IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40), IL-12 (p70), IL-15, IL-16, IP-10, MCP-1, MCP-2, MCP-3, MCP-4, MCSF, MIG, MIP-1 α , MIP-1 β , NAP-2, PDGF, PF-4, PARC, SCF, SDF-1 α , TIMP-1, TIMP-2, TNF β , TGF β , TGF β 2, TGF β 3, VEGF).

Storage

Kit reagents may be stored for up to 6 months at 2-4°C from the date of shipment. Standard vials (recombinant protein) should be stored at -20°C or -80°C (recommended) after reconstitution. Opened Microplate Wells or Reagents may be stored for up to 1 month at 2-4°C. Return unused wells to the pouch containing dessicant pack, reseal along entire edge. Note: the kit can be used within one year if the whole kit is stored at -20°C. **Avoid repeated freeze-thaw cycles.**



Reagents and materials supplied in the kit:

Items	Quantity
A. Microplate coated with Anti-Human TGF- β 1	1 x 96 wells (12 strips of 8 wells)
B. Wash Buffer Concentrate (20x)	25 ml
C. Recombinant Human TGF- β 1 Standards	2 vials
D. Assay Diluent A: Standard/Test Sample Diluent for Serum and Plasma *(contains 0.09% sodium azide as a preservative)	30 ml
E. Assay Diluent B (5x): Standard/Test Sample Diluent for Cell Culture Supernatants and Urine	15 ml
F. Detection Antibody for Human TGF- β 1 Biotinylated anti-human TGF- β 1 (each vial is sufficient to assay half a microplate)	2 vials
G. Streptavidin-HRP Concentrate (25,000x)	8 μ l
H. TMB One-Step Substrate Reagent: 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution	12 ml
I. Stop Solution: 2 M Sulfuric Acid	8 ml



****Precaution: Sodium Azide is a poisonous and hazardous substance which should be handled by trained staff only.***



Additional Materials / Reagents required but not provided:

Items
1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 μ l to 1 ml volumes
3. Adjustable 1-25 ml pipettes for reagent preparation
4. 100 ml and 1 liter graduated cylinders
5. Absorbent paper
6. Distilled or deionized water
7. Log-log graph paper or computer and software for ELISA data analysis
8. Tubes to prepare standard or sample dilutions



Preparation of Kit Reagents

Bring all reagents and samples to room temperature (18-25°C) before use.

Sample Dilution

If your samples need to be diluted, use Assay Diluent A (Item D) for dilution of serum/plasma samples, and Assay Diluent B (Item E) for dilution of culture supernatants and urine.

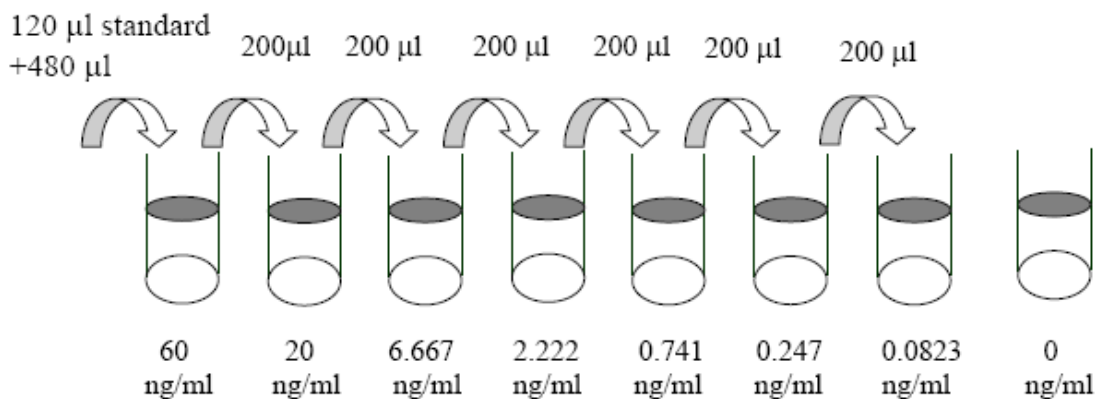
Assay Diluent B

Dilute 5-fold with deionized or distilled water.

Preparation of TGF- β 1 Standard

- Briefly spin the vial of Item C (Recombinant Human TGF- β 1 Standard).
- Add 400 μ l Assay Diluent A (Item D, for serum/plasma samples) or 1x Assay Diluent B (Item E, for cell culture supernatants and urine) to prepare a 300 ng/ml standard. Dissolve the powder thoroughly by gentle mixing.
- Add 120 μ l TGF- β 1 Standard from the vial of Item C, into a tube with 480 μ l Assay Diluent A or 1x Assay Diluent B to prepare a 60 ng/ml stock standard solution.
- Pipet 400 μ l Assay Diluent A or 1x Assay Diluent B into each tube to produce a dilution series indicated in Figure 1 below.
- Mix each tube thoroughly before the next transfer. Gently vortex to mix.
- Assay Diluent A or B serves as the zero standard (0 ng/ml).

Figure 1



Wash Buffer Concentrate

- If the Wash Concentrate (Item B) contains crystals, warm to RT and mix gently until dissolved
- Dilute 20 ml of Wash Buffer Concentrate into de-ionized or distilled water to yield 400 ml of 1x Wash Buffer.



Detection Antibody

- Briefly spin Detection Antibody vial (Item F) before use.
- Add 100 μ l of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate.
- Pipette up and down to gently mix (the concentrate can be stored at 2-4°C for 5 days).
- The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of the **ELISA Method**.

Streptavidin-HRP Concentrate

- Briefly spin Streptavidin-HRP Concentrate vial (Item G), pipette up and down to mix gently before use.
- Streptavidin-HRP concentrate should be diluted 25,000-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 2 μ l of Streptavidin-HRP concentrate into a tube with 198.0 μ l 1x Assay Diluent B to prepare a 100-fold diluted Streptavidin-HRP solution (do not store the diluted solution for next day use). Mix thoroughly and then pipet 60 μ l of prepared 100-fold diluted solution into a tube with 15 mL 1x Assay Diluent B to prepare a final 25,000 fold diluted Streptavidin-HRP solution.

Preparation of Reagents to activate Cell Culture Supernatants/Urine Samples and Serum/Plasma Samples

1 N HCl (100 ml)

Slowly add 8.33 ml of 12 N HCl into 91.67 ml deionized water. Mix bottle.

0.5 M HEPES + 1.2 N NaOH (100 ml)

Slowly add 12 ml of 10 N NaOH into 75 ml deionized water. Mix bottle. Add 11.9 g HEPES. Mix thoroughly. Bring final volume to 100 ml with deionized water.

2.5 N Acetic Acid + 10 M Urea (250 ml)

Add 150.2 g of Urea into 100 ml deionized water. Mix bottle until dissolved. Slowly add 35.9 ml of Glacial Acetic Acid. Mix thoroughly. Bring final volume to 250 ml with deionized water.

1 M HEPES + 2.7 N NaOH (250 ml)

Add 67.5 ml of 10 N NaOH into 140 ml deionized water. Mix bottle. Add 59.5 g HEPES. Mix through. Bring final volume to 250 ml with deionized water.

TGF- β 1 SAMPLE ACTIVATION PROCEDURE

To activate latent TGF- β 1 to the immunoreactive form, follow the activation procedure outlined below. Assay samples after neutralization (pH 7.0 - 7.6). Use polypropylene test tubes.

Notes: Do not activate the kit standards. The kit standards contain active rhTGF- β 1.



Cell Culture Supernatants/Urine

Add 0.1 ml 1 N HCl into 0.5 ml cell culture supernatants or urine. Mix tube thoroughly. Incubate for 10 minutes at room temperature. Neutralize the acidified sample by adding 0.1 ml 0.5 M HEPES + 1.2 N NaOH (pH 7.0-7.6).

Mix tube thoroughly. Assay immediately. The activated sample may be diluted with 1x Assay Diluent B (for cell culture supernatants/urine). The concentration read off the standard curve must be multiplied by the dilution factor.

Serum/plasma

Add 0.1 ml 2.5 N Acetic Acid + 10 M Urea to 0.1 ml serum. Mix tube thoroughly. Incubate for 10 minutes at room temperature. Neutralize the acidified sample by adding 0.1 ml 1 M HEPES + 2.7 N NaOH. Mix tube thoroughly. Assay immediately. The activated sample may be further diluted 3 fold with Assay Diluent A. The concentration read off the standard curve must be multiplied by the dilution factor.

ELISA Method:

Be sure to read 'Preparation of Kit Reagents' before carrying out the assay

1. Bring all reagents and samples to room temperature (18-25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard (see **Preparation of Kit Reagents: TGF-β1 Standard**) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature or overnight at 2-4°C.
3. Discard the solution and wash 4 times with 1x Wash Solution (300 µl each). Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of 1x prepared biotinylated antibody (see **Preparation of Kit Reagents: Detection Antibody**) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution and wash 4 times with 1x Wash Solution (300 µl each) as in step 3.
6. Add 100 µl of prepared Streptavidin solution (see **Preparation of Kit Reagents: Streptavidin-HRP Concentrate**) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution and wash 4 times with 1x Wash Solution (300 µl each) as in step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µl of Stop Solution (Item I) to each well. Read immediately at 450 nm.



ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.



2. Add 100 μ l standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.



3. Add 100 μ l prepared biotinylated antibody to each well. Incubate 1 hour at room temperature.



4. Add 100 μ l prepared Streptavidin solution. Incubate 45 minutes at room temperature.



5. Add 100 μ l TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.



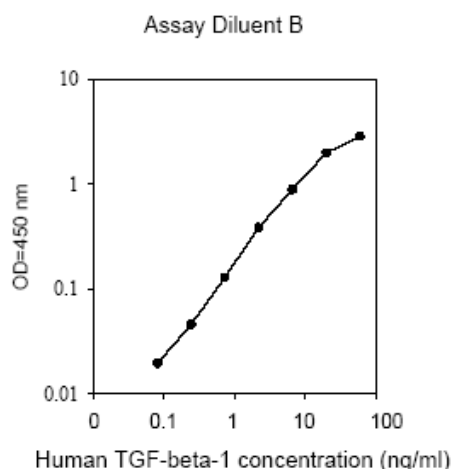
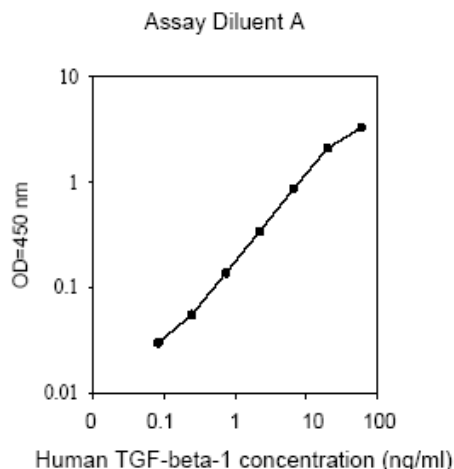
6. Add 50 μ l Stop Solution to each well. Read immediately at 450 nm.

CALCULATION OF RESULTS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

TYPICAL DATA

These standard curves are for demonstration only. A standard curve must be run with each assay.



Troubleshooting Guide:

Problem	Cause	Solution
1. Poor Standard Curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times	1. Ensure sufficient incubation time; change ELISA Method procedure Step 2 to overnight.
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation.
3. Large CV	1. Inaccurate pipetting	1. Check pipettes.
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer.
5. Low sensitivity	1. Improper storage of the ELISA Kit	1. Store your standard at <-20°C after reconstitution, others at 2-4°C. Keep substrate solution protected from light.
	2. Stop solution	2. Stop solution should be added to each well before measure.

NOT FOR HUMAN USE. FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.



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