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Human Transforming Growth Factor alpha ELISA Kit

Catalog No: CKH188 **Size:** 1 x 96 tests

Introduction:

Transforming Growth Factors (TGFs) are biologically active polypeptides that reversibly confer the transformed phenotype on cultured cells. TGF alpha (TGFA) shows about 40% sequence homology with Epidermal Growth Factor (EGF) and competes with EGF for binding to the EGF Receptor (EGFR), stimulating its phosphorylation and producing a mitogenic response.

The Human TGF alpha ELISA is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human TGF alpha in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for TGF alpha coated on a 96-well plate. Standards and samples are pipetted into the wells and TGF alpha present in a sample is bound to the wells by the immobilized antibody. The wells are washed and Biotinylated Anti-Human TGF alpha antibody is added. After washing away unbound Biotinylated antibody, HRP-Streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of TGF alpha bound.

Performance and Characteristics:

Sensitivity

The minimum detectable dose of TGF alpha is typically less than 3 pg/mL.

Reproducibility

Intra-Assay: CV<10%
Inter-Assay: CV<12%

Recovery

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

Sample Type	Average % Recovery	Range (%)
Serum	92.34	82-104
Plasma	93.46	83-103
Cell culture media	94.49	84-105

Linearity

Sample Type		Serum	Plasma	Cell culture media
1:2	Average % of Expected	91	93	92
	Range (%)	83-103	84-105	83-103
1:4	Average % of Expected	94	90	93
	Range (%)	82-102	83-104	84-104
1:8	Average % of Expected	92	94	95
	Range (%)	84-103	82-102	83-102

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Reagents and materials supplied in the kit:

Items	Quantity
A. Microplate coated with Anti-Human TGF alpha	96 wells
B. Wash Buffer Concentrate (20x)	25 mL
C. Recombinant Human TGF alpha Standards	2 vials
D. Assay Diluent A: Standard/Sample-Serum/Plasma *	30 mL
E. Assay Diluent B (5x): Standard/Sample-Cell Culture Medium/Urine	15 mL
F. Detection Antibody: Anti-Human TGF alpha	2 vials
G. Streptavidin-HRP Concentrate (15,000x)	8 µl
H. TMB One-Step Substrate Reagent (TMB in buffered solution)	12 mL
I. Stop Solution (2 M Sulfuric Acid)	8 mL

** Contains 0.09% Sodium Azide as preservative. Precaution: Sodium Azide is a poisonous and hazardous substance which should be handled by trained staff only.*

Storage of Kit Reagents:

Stable for up to 6 months from date of shipment at 2-4°C. Store reconstituted standard (recombinant protein) at -80°C. Opened Microplate Wells and reagents are stable for 1 month at 2-4°C. Return unused wells to the pouch containing desiccant pack and reseal along the entire edge.

Materials/reagents required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µl to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- 100 mL and 1 liter graduated cylinders
- Absorbent paper
- Distilled or deionized water
- Log-log graph paper or computer/software for data analysis
- 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.

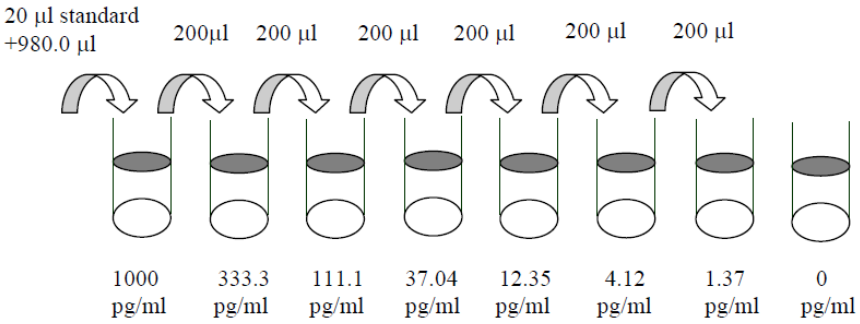
Wash Buffer Concentrate

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

TGF alpha Standard

- Briefly spin the vial of Item C (Recombinant Human TGF alpha Standard).
- Add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix.
- Add 6 µl standard from the vial of Item C, into a tube with 744 µl Assay Diluent A or B to prepare a 400 pg/mL stock standard solution.
- Pipet 450 µl Assay Diluent A or 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 pg/mL).

Figure 1



Detection Antibody

- Briefly spin Detection Antibody vial (Item F) before use.
- Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate.
- Mix gently (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 15,000-fold with 1x Assay Diluent B.

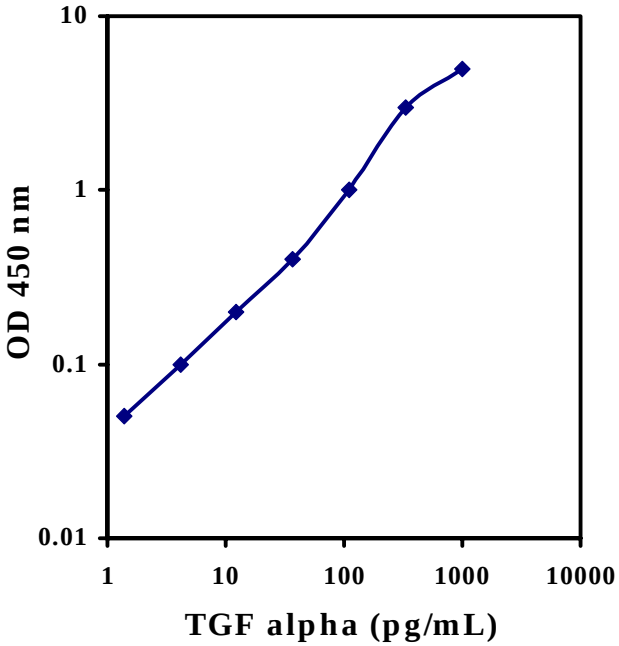
For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 2 µl of Streptavidin-HRP concentrate into a tube with 198.0 µl 1x Assay Diluent B to prepare a 100-fold diluted Streptavidin-HRP solution (do not store the diluted solution for next day use). Mix thoroughly and then pipet 50 µl of prepared 100-fold diluted solution into a tube with 15 mL 1x Assay Diluent B to prepare a final 15,000 fold diluted Streptavidin-HRP solution.

Assay Procedure Summary:

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at RT or overnight at 2-4°C.
3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at RT.
4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at RT.
5. Add 100 µl TMB to each well. Incubate 30 minutes at RT.
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

Typical Data:

Standard curve is for demonstration **ONLY**. A standard curve **MUST** be run with each assay.



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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 alpha Standard**) and sample into appropriate wells. Cover well 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).

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.

5. Discard the solution and wash 4 times with 1x Wash Solution (300 µl each).

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.

7. Discard the solution and wash 5 times with 1x Wash Solution (300 µl each).

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.

9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

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.

Troubleshooting Guide:

Problem	Cause	Solution
1. 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 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	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.

Disclaimer - This information is believed to be correct, but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

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

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