Datasheet for ABIN625094

TGFB1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	TGFB1
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	18 pg/mL - 4000 pg/mL
Minimum Detection Limit:	18 pg/mL
Application:	ELISA

Product Details

Purpose:	Human TGF beta 1 ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with any of the cytokines tested: Human ANG, CD23, Eotaxin, GCSF, GM-CSF, GRO-alpha, GRO-beta, GRO-gamma, I-309, IFN-gamma, IL-1 alpha, IL-1 beta, IL-3, IL-4, IL-5, IL-6, IL-7
Sensitivity:	18 pg/mL
Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments • Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data

Product Details

Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Stop Solution • Assay Diluent(s) • Lyophilized Standard • Biotinylated Detection Antibody • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 µL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • Log-log graph paper or computer and software for ELISA data analysis

Target Details

Target:	TGFB1
Alternative Name:	TGF-beta 1 (TGFB1 Products)
Background:	Transforming Growth Factor Beta (TGF-beta) is a stable, multifunctional polypeptide growth factor. TGF-beta exists in at least five isoforms, known as TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5. Their amino acid sequences display homologies on the order of 70-80%. The various TGF-beta 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-beta1 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. The Human TGF-beta1 ELISA (Enzyme-Linked mmunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human TGF-beta1 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human TGF-beta1 coated on a 96-well plate. Standards and samples are pipetted into the wells and TGF-beta1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human TGF-beta1 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated 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-beta1 bound. The Stop Solution

Target Details

	changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.
Gene ID:	7040
UniProt:	P01137
Pathways:	EGFR Signaling Pathway, Dopaminergic Neurogenesis, Cellular Response to Molecule of Bacterial Origin, Glycosaminoglycan Metabolic Process, Regulation of Leukocyte Mediated Immunity, Regulation of Muscle Cell Differentiation, Positive Regulation of Immune Effector Process, Cell-Cell Junction Organization, Production of Molecular Mediator of Immune Response, Ribonucleoside Biosynthetic Process, Skeletal Muscle Fiber Development, Regulation of Carbohydrate Metabolic Process, Protein targeting to Nucleus, Autophagy, Cancer Immune Checkpoints

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples3 fold after treatment (see activation steps on page 6)
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C. 4. Add 100 µL of prepared biotin antibody to each well. 5. Incubate 1 h at RT. 6. Add 100 µL of prepared Streptavidin solution to each well. 7. Incubate 45 min at RT. 8. Add 100 µL of TMB One-Step Substrate Reagent to each well. 9. Incubate 30 min at RT. 10. Add 50 µL of Stop Solution to each well. 11. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18-25 °C) before use. 2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of culture supernatants and urine. 3. Assay Diluent B should be diluted 5-fold with deionized or distilled water. 4. Preparation of standard: Briefly spin the vial of Item C. Add 700 µL Assay Diluent A (Item D)

(for serum/plasma) or 1x Assay Diluent B (Item E) (for cell culture supernatants/urine) to prepare a 300 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 120 µL TGF-beta1 standard from the vial of Item C, into a tube with 480 µL Assay Diluent A or 1x Assay Diluent B to prepare a 60 ng/mL stock standard solution. Pipette 400 µL Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 ng/mL). 200 µL 120 µL standard +480 µL 200myl 200 µL 200 µL 200 µL 200 µL 60 20 6.667 2.222 0.741 0.247 0.0823 0 ng/mL ng/mL ng/mL ng/mL ng/mL ng/mL ng/mL ng/mL

5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 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 Part VI Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin concentrate should be diluted 500-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 20 µL of HRP-Streptavidin concentrate into a tube with 10 ml 1x Assay Diluent B to prepare a 500-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well. *Reagents to activate cell culture supernate/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. 1.2 N NaOH/0.5 M HEPES (100 ml) - Slowly add 12 ml of 10 N NaOH into 75 mL deionized water. Mix bottle. Add 11.9 g HEPES. Mix through. 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 through. Bring final volume to 250 ml with deionized water. 2.7 N NaOH/1 M HEPES (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. VI. TGF-beta1 SAMPLE ACTIVATION PROCEDURE To activate latent TGF-beta1 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-beta1. 1. Cell Culture Supernates/Urine Add 0.1 ml 1 N HCl into 0.5 mL cell culture supernate or urine. Mix tube thoroughly. Incubate for 10 minutes at room temperature. Neutralize the acidified sample by adding 0.1 ml 1.2 N NaOH/0.5 M HEPES (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. 2. 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 2.7 N NaOH/1 M HEPES. Mix tube thoroughly. Assay immediately. The activated sample may be diluted with Assay Diluent A. The concentration read off the standard curve must be multiplied by the dilution factor.
Assay Procedure:	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 Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 myl) 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 (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 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 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. Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Assay Diluent A Human TGF-beta-1 concentration (ng/mL) O D =4 50 n m 0.01 0.1 1 10 0 0.1 1 10 100 Assay Diluent B Human TGF-beta-1 concentration (ng/mL) O D =4 50 n m 0.01 0.1 1 10 0 0.1 1 10 100 Sensitivity: The minimum detectable dose of TGF-beta1 is typically less than 80 pg/mL. Recovery: Recovery was determined by spiking various levels of human TGF-beta1 into human

Application Details

	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 Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %
Assay Precision:	Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months

Publications

Product cited in:	Passanha, Geuens, LaPointe: "Cadherin-11 influences differentiation in human mesenchymal stem cells by regulating the extracellular matrix via the TGFβ1 pathway." in: **Stem cells (Dayton, Ohio)**, (2022) (PubMed). Prestigiacomo, Suter-Dick: "Nrf2 protects stellate cells from Smad-dependent cell activation." in: **PLoS ONE**, Vol. 13, Issue 7, pp. e0201044, (2019) (PubMed). Xing, Xiao, Lu, Zhu, He, Huang, Lopez, Wong, Ju, Tian, Zhang, Xu, Wang, Li, Karin, Ren: "GFI1 downregulation promotes inflammation-linked metastasis of colorectal cancer." in: **Cell death and differentiation**, Vol. 24, Issue 5, pp. 929-943, (2018) (PubMed). Jha, Mathur, Svedlund, Ye, Yeghiazarians, Healy: "Molecular weight and concentration of heparin in hyaluronic acid-based matrices modulates growth factor retention kinetics and stem cell fate." in: **Journal of controlled release : official journal of the Controlled Release Society**,

Vol. 209, pp. 308-16, (2016) (PubMed).

Khoo, Nadarajan, Shim, Miswan, Zang, Possinger, Elstner: "Pretreatment of BMSCs with TZD solution decreases the proliferation rate of MCF-7 cells by reducing FGF4 protein expression." in: **Molecular medicine reports**, Vol. 13, Issue 4, pp. 3406-14, (2016) (PubMed).

There are more publications referencing this product on: Product page

Images

ELISA

Image 1.