Science for Society

Human Transforming Growth Factor Beta 3 (TGF-β3) ELISA Kit

to determine Transforming Growth Factor Beta 3 ELISA Kit in Serum, Blood Plasma, Saliva, Urine, And Other Related Tissue Liquid Samples.

INSTRUCTION MANUAL

FOR ELISA KIT No: LTuH6892EA

Arsh Biotech Pvt. Ltd.

308, Aggarwal City Mall, Road No.44,
Pitampura, Delhi-110034, India
Toll Free: 1800-3000-8822
Mobile: +91-98105-21400 | Fax: +91-11-42208444
info@arshbiotech.com

INTENDED USE

This kit is used to assay the Transforming Growth Factor Beta 3 (TGF-β3) in the sample of serum, blood plasma and other related biological liquid. For *in vitro* use only.

MANUAL VERSION 1.01

Science for Society

PRINCIPLE

This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human Transforming Growth Factor Beta 3. Transforming Growth Factor Beta 3 (TGF-β3) is added to the wells, which are pre-coated with Transforming Growth Factor Beta 3 (TGF-β3) monoclonal antibody. This is followed by addition of anti- TGF-β3 antibodies labeled with biotin which are further allowed to unite with streptavidin-HRP and form an immune complex. Unbound proteins are removed after incubation by washing and substrate A and B are added to the wells. The solution turns blue and changes into yellow with the effect of acid. The optical density of the solution and the concentration of Transforming Growth Factor Beta 3 (TGF-β3) are positively correlated.

MATERIALS SUPPLIED IN THIS KIT

1	Standard solution (2400ng/L)	0.5ml	7	Chromogen solution A	6ml
2	Standard dilution solution	3ml	8	Chromogen solution B	6ml
3	Coated ELISA plate	12X8 Strips	9	Stop solution	6ml
4	Streptavidin-HRP	6ml	10	Instruction	1
5	Washing buffer concentrate (30X)	20ml	11	Seal plate membrane	2
6	Anti-human TGF-β3 Antibody labeled with biotin	1ml	12	Zipper bag	1

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips

4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

Science for Society

IMPORTANT NOTES

1. Before using, keep the kit outside and allow it to come to room temperature.
2. After breaking the seal of ELISA coated-plate, keep the unused strips in the zipper bag at 2-8 °C.
3. Pipette tips and seal plate membrane should not be used more than once in order to avoid cross contamination.
4. All samples and all discard generated should be disposed as per local rules.
5. Reagents of different batches must not be mixed and should be used before their respective validity dates.
6. Substrate B is sensitive to light and therefore should not be exposed to light for too long.

PRECISION

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level TGF-β3 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level TGF-β3 were tested on 3 different plates, 8 replicates in each plate.

CV(%) = SD/meanX100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

SAMPLE PREPARATION

1.Samples containing NaN_3 are not recommended for testing with ELISA as they may inhibit the activity of Horse Radish Peroxidase (HRP).

2.After extraction, experiment should be conducted immediately. Otherwise, keep the sample at -20℃. Avoid repeated freeze-thaw cycles.

3.Serum: Allow the sample to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RCF) for 20 minutes. Collect the supernatant carefully. In case of storage, centrifugation should be performed again prior to use.

4.Blood plasma: During sample collection EDTA or sodium citrate should be used for anti-coagulation. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

5.Urine: Collect the sample in a sterile tube. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow a similar procedure.

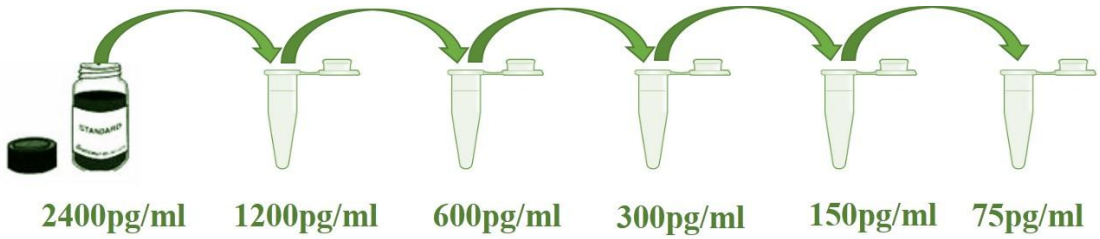
6.Cell culture supernatant: For secreted components, centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells by repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

7.Tissue sample: Incise tissue sample of interest and add few mls of PBS (pH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8℃. Add few mls of PBS (pH 7.4) and then homogenize the sample thoroughly by hand or by homogenizer. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

ASSAY PROCEDURE

1. Dilution of standard solutions: This kit contains a standard of known concentration, which could be diluted in small tubes by the end-user by following the instruction in the table below:

1200ng/	Standard No.5	120µl Original Standard + 120µl Standard diluent
600ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard diluent
300ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
150ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
75ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard diluent



2. The number of strips needed is determined by the number of samples to be tested added by the number of standards to be run by the end-user plus a blank control well. It is recommended to run all wells in duplicate or triplicates.

3.Sample Addition:

A) Blank well: Contains no sample, no anti TGF-β3 antibody labeled with biotin and no streptavidin-HRP. Nothing is added to the blank well at this step.

B) Standard solution well: Add 50µl standard and 50µl streptavidin-HRP (please note: biotin antibodies are already present in the standard solution so no biotin antibodies are added to these wells).

C) Sample well to be tested: Add 40µl sample and then add 10µl anti- TGF-β3 biotin antibody, and then add 50µl streptavidin-HRP in a step-wise manner. Then cover it

with seal plate membrane. Shake gently to mix the wells and incubate at 37℃ for 60 minutes.

4. Preparation of washing solution: Dilute the washing concentrate (30X) with distilled water.

5. Washing: Remove the seal plate membrane carefully, drain the liquid in the wells and shake off the remaining liquid by tapping on an absorbent paper. Fill each well with washing solution. Drain the liquid after 30 seconds of soaking. Then repeat this procedure five times and blot the plate.

6. Color development: Add 50µl chromogen solution A firstly to each well. Then add 50µl chromogen solution B to each well. Shake gently to mix the wells and incubate for 10 minutes at 37℃ away from light for color development.

7. Stop: Add 50µl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment).

8. Assay: Set blank well as zero absorbance and measure the absorbance (OD) of each well at 450nm wavelength using a microplate reader. This reading should be taken within 10 minutes after having added the stop solution.

9. Based on the observed OD values for the various standards, calculate the linear regression equation of the standard curve. Then as per the OD value of test samples, calculate the concentration of the corresponding sample.

PROTOCOL SUMMARY

Prepare reagents, samples and standards.

↓

Add prepared samples and standards in respective wells together with second antibody labeled with biotin and ELISA solutions as instructed. Incubate for 60 minutes at 37℃.

↓

Wash the plate five times. Add Chromogen solution A and B. Incubate for 10 minutes at 37 ℃ for color development.

↓

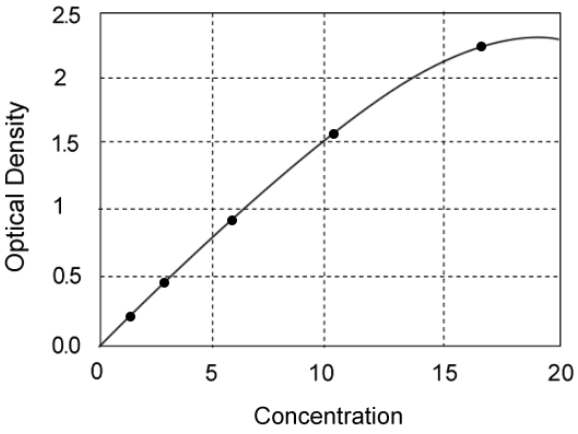
Add stop solution

↓

Read the OD value within 10minutes.

↓

Plot and Calculate.



THE CHART SHOWN HERE IS FOR REFERENCE PURPOSES ONLY AND THE ACTUAL PERFORMANCE MAY VARY.

CALCULATION

Plot the concentration of standards on the abscissa and OD value on the ordinate axis. Draw the best fitting standard curve. Based on the OD of the test sample, locate its corresponding concentration (which is the concentration of the sample) on the graph; or alternatively calculate the linear regression equation of standard curve according to the concentration of the standard and the OD values. Then substitute with the OD value of the test sample to calculate its concentration.

ASSAY RANGE： 5ng/L → 2000ng/L

SENSITIVITY：<2.51ng/L

PACKAGE SIZE：96Tests.

VALIDITY & STORAGE: Six months (at 2-8℃) or Twelve months (at -20℃) from date of purchase. Avoid repeated freeze thaw cycles.