BioOcean® Rat TGF-β1 ELISA Kit

Catalog Number EK381
Size: 96 Test

For the quantitative determination of rat transforming growth factor beta 1 (TGF-β1) concentrations in cell culture supernates, serum and plasma.

This package insert must be read entirely before using this product. For proper performance, follow the protocol provided with each individual kit.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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QUICK PROCEDURE FLOWCHART

1. Prepare all reagents and standards as directed.

2. Add 50 µl *Assay Buffer* to each well.

3. Add 50 µl *Standard* or sample per well within 15 minutes. Incubate for 2 hours at RT.

4. Aspirate and wash 6 times.

5. Add 100 µl *Detect Antibody* to each well. Incubate for 2 hours at RT.

6. Aspirate and wash 6 times.

7. Add 100 µl *Streptavidin-HRP* to each well. Incubate for 45 minutes at RT.

8. Aspirate and wash 6 times.

9. Add 100 µl *Substrate Solution* to each well.

Incubate for 5 - 30 minutes at RT. Protect from light.

10. Add 100 µl *Stop Solution* to each well.

11. Read at 450 nm within 30 minutes. Correction 570 or 630 nm.

DESCRIPTION

Transforming growth factor beta 1 (TGF-β1) is a polypeptide member of the transforming growth factor beta superfamily of cytokines that performs many cellular functions, including the control of cell growth,cell proliferation, cell differentiation and apoptosis. TGF-βs are a multifunctional set peptides that controlsproliferation, differentiation, and other functions in many cell types. TGF-βs act synergistically with TGFA in inducingtransformation. It also acts as a negative autocrine growth factor. Dysregulation of TGF-β activation and signaling may result in apoptosis. Many cells synthesize TGF-β and almost all of them have specific receptors for this peptide. TGF-β1, TGF-β2 and TGF-β3 all function through the same receptor signaling systems. TGF-β1 plays an important role in controlling the immune system, and shows different activities on different types of cell, or cells at different developmental stages. Most immune cells (orleukocytes) secrete TGF-β1.

TGF-β1 is related to cancer, autoimmune diseases, liver diseases, kidney diseases, diabetes, cardiovascular diseases, asthma, chronic obstructive pulmonary disorder (COPD), cystic fibrosis (CF) and so on.

PRINCIPLE OF THE ASSAY

BioOcean Rat TGF-β1 ELISA Kit is based on the quantitative sandwich enzyme-linked immunosorbent assay technique to measure concentration of rat TGF-β1 in the samples. A monoclonal antibody specific for rat TGF-β1 has been immobilized onto microwells. Standard or samples are pipetted into the wells, and TGF-β1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-linked monoclonal antibody specific for TGF-β1 is added to the wells. Following a wash to remove any unbound detect antibody, streptavidin-HRP is added. After washing, substrate solution reacts with HRP and color develops in proportion to the amount of TGF-β1 bound by the immobilized antibody. The color development is stopped by addition of acid and the optical density value is measured by microplate reader.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use expired kit or reagents.
- Do not use reagents from other lots or manufacturers. Do not prepare component by yourself.
- If concentration of assayed factor in samples is higher than the highest standard, dilute the serum/plasma samples with *Assay Buffer*, dilute the cell culture supernate samples with *cell culture medium*. Reanalyze these and multiply results by the appropriate dilution factor.
- Any variation in testing personnel, sample preparation, standard dilution, pipetting technique, washing techniques, incubation time, temperature, kit age and equipment can cause variation in results.
- Bacterial or fungal contamination in either samples or reagents, or cross-contamination between reagents may cause erroneous results.

MATERIALS PROVIDED

Unopened kit should be stored at 2 - 8℃.

- **TGF-β1 Microplate** (1 plate): 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against rat TGF-β1.
- **TGF-β1 Standard** (2 vials): Recombinant rat TGF-β1 in a buffered protein base with preservatives; lyophilized.
- **TGF-β1 Detect Antibody** (1 vial, 120 μl): Biotin-conjugate anti-rat TGF-β1 monoclonal antibody; 100× liquid.
- **Streptavidin-HRP** (1 vial, 150 μl): 100× liquid.
- **Assay Buffer (10×)** (1 bottle, 10 ml): PBS with 0.5 % Tween-20 and 5 % BSA.
- **Substrate** (1 bottle, 15 ml): TMB (tetramethyl-benzidine).
- **Stop Solution** (1 bottle, 15 ml): 0.18 M sulfuric acid.
- **Washing Buffer (20×)** (1 bottle, 50 ml): PBS with 1 % Tween-20.
- **Plate Covers** (5 strips).
- **HCl** (1 bottle, 3 ml): 1N.
- **NaOH** (1 bottle, 3 ml): 1N.

STORAGE

Store kit reagents between 2 and 8℃. Immediately after use remaining reagents should be returned to cold storage (2 to 8℃). Expiry of the kit and reagents is stated on labels.
Expiration date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

<table>
<tr><td colspan="2">Unopened kit</td><td>Store at 2 - 8℃ (See expiration date on the label).</td></tr>
<tr><td rowspan="3">Opened/
Reconstituted
Reagents</td><td>1× Washing Buffer
1× Assay Buffer
Stop Solution
Substrate TMB
Detect Antibody
Streptavidin-HRP</td><td>Up to 1 month at 2 - 8℃.</td></tr>
<tr><td>Reconstituted Standard</td><td>Up to 1 month at ≤ -20℃ in a manual defrost freezer. Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Microplate Wells</td><td>Up to 1 month at 2 - 8℃. Return unused strips to the foil pouch containing the desiccant pack, reseal along entire edge to maintain plate integrity.</td></tr>
</table>

Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- **Microplate reader** capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
- **Pipettes and pipette tips**.
- 50 μl to 300 μl adjustable **multichannel micropipette** with disposable tips.
- Multichannel micropipette **reservoir**.
- **Beakers, flasks, cylinders** necessary for preparation of reagents.
- **Deionized or distilled water**.
- **Polypropylene** test tubes for dilution.

PRECAUTION

- Intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Treat all chemicals with caution because they can be potentially hazardous.
- It is recommended that this product is handled only by persons who have been trained in laboratory techniques and in accordance with the principles of good laboratory practice. Wear personal protection equipment such as laboratory coat, safety glasses and gloves.
- Avoid direct contact with skin or eyes. Wash immediately with water in the case of contact with skin or eyes. Avoid contact of skin or mucous membranes with kit reagents or specimens. See material safety data sheet(s) for specific advice.
- Pure water or deionized water must be used for reagent preparation.
- The Stop Solution provided with this kit is an acid solution. Wear personal protection equipment with caution.
- Do not expose kit reagents to strong light during storage and incubation.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- Use disposable pipette tips and/or pipettes to avoid microbial or cross-contamination of reagents or specimens which may invalidate the test.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the HRP and antibody conjugate.
- Substrate solution must be warmed to room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5℃.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

TECHNICAL HINTS

- All reagents including microplate, samples, standards and working solution should be warmed to room temperature before use.
- To obtain accurate results, using adhesive film to seal the plate during incubation is suggested.
- It is recommended that all samples and standards be assayed in duplicate.
- Avoid foaming when mixing or reconstituting solutions containing protein.
- To avoid cross-contamination, use separate reservoirs for each reagent and change pipette tips between each standard, sample and reagent.
- When using an automated plate washer, adding a 30 seconds soak period before washing step and/or rotating the plate between wash steps may improve assay precision.
- When pipetting reagents, maintain a consistent order of addition from well-to-well.
- Keep Substrate solution protected from direct strong light. Substrate Solution should turn to gradations of blue after a proper color development.
- Read absorbance within 30 minutes after adding stop solution.
- Take care not to scratch the inner surface of the microwells.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates – Remove particulates by centrifugation and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

Serum – Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000 g. Remove serum and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

Urine – Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately or aliquot and store at at ≤ -20℃. Avoid repeated freeze-thaw cycles.

Note: Neat unactivated urine samples exhibit a decrease in TGF-β1 concentration in the first 24 hours of storage (frozen or refrigerated). Care should be taken that samples are assayed under identical storage conditions and durations.

Plasma – Collect plasma using EDTA as anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. An additional centrifugation step of the plasma at 10000 g for 10 minutes at 2 - 8℃ is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

TGF-β1 is present in platelet granules and is released upon platelet activation. Therefore, to measure circulation levels of TGF-β1, platelet-poor plasma should be collected for measurement. It should be noted that many protocols for Clinical Laoratory Standards (NCCLS), result in incormplete removal of platelet from blood. This will cause variable and irreproducible results for assays of factors contained in platelet and released by platelet activation. The recommended plasma collection protocol is designed to minimize platelet degranulation. However, since even the best methods for plasma collection may result in some platelet degranulation on occasion.

Note: Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

CELL CULTURE SUPERNATE NOTE

Significant levels of latent TGF-β1 are found in bovine, porcine, equine, and caprine sera. The reported levels of TGF-β1 in bovine and fetal bovine sera can be as high as 16 ng/ml after activation. Therefore, conditioned medium containing 10 % fetal bovine serum can be expected to have a TGF-β1 concentration of about 1600 pg/ml. the background level of TGF-β1 in control medium can be determined and subtracted from samples of conditioned medium. As an alternative, the background level of TGF-β1 in medium can be lowered using the medium containing 10 % serum, the medium is changed of medium over 12 - 24 hours. Cells are then switched to medium alone or medium containing 200 μg/ml crystalline BSA. Particular cell lines may require specific additions to the serum-free medium for maintenance. After 24 hours, the serum-free conditioned medium is clarified by centrifugation and samples are stored at ≤ -20℃. Optionally, 2 μg/ml aprotinin, leupeptin, pepstatin A, and 120 μg/ml PMSF can be added before freezing. Thawed or fresh samples of serum-free or serum-containing conditioned media exceeds 5%, further dilute the activated sample.

SAMPLE ACTIVATION

To activate latent TGF-β1 to immunoreactive TGF-β1, follow the activation procedure outlined below. Assay samples after neutralization (pH 7.2 - 7.6). Use polypropylene test tubes.

Note: Do not activate the kit standards. The kit standards contain active recombinant TGF-β1.

Cell culture Supernates/Urine	Serum/Plasma
100 μl sample + 20 μl 1 N HCl	40 μl sample + 20 μl 1 N HCl
Mix well	Mix well
Incubate 10 minutes at RT	Incubate 10 minutes at RT
Neutralize: + 20 μl 1 N NaOH	Neutralize: + 20 μl 1 N NaOH
Mix well	Mix well
Assay immediately	Dilution: Serum: Active 20 μl + 480 μl Assay Buffer (1×) Plasma: Active 80 μl + 80 μl Assay Buffer (1×)
The concentration read of the standard curve must be multiplied by the dilution factor, final 2.8.	The concentration read of the standard curve must be multiplied by the appropretate dilution factor. Serum: final 100 Plasma: final 8

Note: Activated serum and EDTA plasma samples may be stored for up to 24 hours at 2 - 8℃ before use. Activated cell culture supernate/urine samples must be assay immediately after activation.

REAGENT PREPARATION

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1×)

Pour entire contents (50 ml) of the **Washing Buffer (20×)** into a clean 1000 ml graduated cylinder.
Bring to final volume of 1000 ml with pure or deionized water.
Mix gently to avoid foaming.
Transfer to a clean wash bottle and store at 2 to 25℃. Washing Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (10 ml) of the **Assay Buffer (10×)** into a clean 100 ml graduated cylinder.
Bring to final volume of 100 ml with distilled water. Mix gently to avoid foaming.
Store at 2 to 8℃. Assay Buffer (1×) is stable for 30 days.

Detect Antibody

Mix well prior to making dilutions.
Make a **1：100** dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1×) in a clean plastic tube as needed.
The diluted Detect Antibody should be used within 30 minutes after dilution.

Streptavidin-HRP

Mix well prior to making dilutions.
Make a **1：100** dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1×) in a clean plastic tube as needed.
The diluted Streptavidin-HRP should be used within 30 minutes after dilution.

Sample Dilution

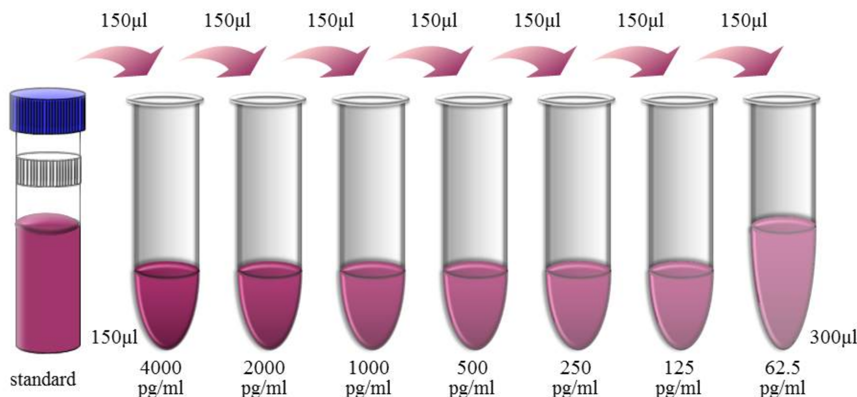
If your samples have high TGF-β1 content, dilute serum/plasma samples with Assay Buffer (1×).
For cell culture supernates, dilute with cell culture medium.

Rat TGF-β1 Standard

Reconstitute **Rat TGF-β1 Standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 8000 pg/ml).
Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.
Use polypropylene tubes.

For serum/plasma samples, mixing *concentrated rat TGF-β1 standard* (150 µl) with 150 µl of *Assay Buffer (1×)* creates the high standard (4000 pg/ml). Pipette 150 µl of *Assay Buffer (1×)* into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. *Assay Buffer (1×)* serves as the zero standard (0 pg/ml).

For cell culture supernates, mixing *concentrated rat TGF-β1 standard* (150 µl) with 150 µl of cell culture medium creates the high standard (4000 pg/ml). Pipette 150 µl of cell culture medium into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium serves as the zero standard (0 pg/ml).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.
2. Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.
3. Add 300 µl *Washing Buffer (1×)* per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess *Washing Buffer (1×)*. Use the microwell strips immediately after washing. **Do not allow wells to dry.**
4. Add 50 µl of *Assay Buffer (1×)* to each well.
5. Add 50 µl of *Standard* or sample per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.
6. Seal the plate with an *adhesive film*. Incubate at room temperature (18 to 25℃) for 2 hours on a microplate shaker set at 300 rpm. (Shaking is absolutely necessary for an optimal test performance.)
7. Aspirate each well and wash by filling each well with 300 µl *Washing Buffer (1×)*, repeat five times for a total six washes. Complete removal of liquid at each step is essential to the best performance. After the last wash, remove any remaining *Washing Buffer (1×)* by aspirating or decanting. Invert the plate and tap it against clean paper towels.
8. Add 100 µl of *Detect Antibody* to each well.
9. Seal the plate with a fresh *adhesive film*. Incubate at room temperature (18 to 25℃) for 2 hours on a microplate shaker set at 300 rpm.
10. Repeat aspiration/wash as in step 7.

11. Add 100 μl of *Streptavidin-HRP* to each well.
12. Seal the plate with a fresh *adhesive film*. Incubate at room temperature (18 to 25℃) for 45 minutes on a microplate shaker set at 300 rpm.
13. Repeat aspiration/wash as in step 7.
14. Add 100 μl of *Substrate Solution* to each well. Incubate for 5 - 30 minutes at room temperature. Protect from light.
15. Add 100 μl of *Stop Solution* to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
16. Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

CALCULATION OF RESULTS

Average the duplicate optical density readings for each standards and sample, then subtract the average optical density value of the zero standard.

Standard Concentration as horizontal axis, optical density (OD) Value as the vertical axis, regressing the data and create a standard curve using computer software. The data may be linearized by plotting the log of the TGF-β1 concentrations versus the log of the OD and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

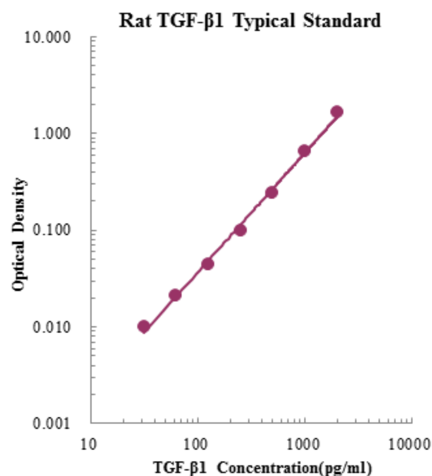
Note: The finally concentration of top standard is 2000 pg/ml. If instruction in this protocol have been followed samples have been diluted by 1:1 ratio (50 μl sample + 50 μl Assay Buffer), the concentration read from the standard curve must be multiplied by the dilution factor (×2).

If samples have been diluted following the instruction, the concentration read from the standard curve must be multiplied by the dilution factor (See Sample Activation).

TYPICAL DATA

A standard curve must be run within each assay. This standard curve is provided for demonstration only.

(pg/ml)	O.D.		Average	Corrected
0.00	0.025	0.025	0.025	
31.25	0.035	0.034	0.035	0.010
62.50	0.046	0.045	0.046	0.021
125.00	0.068	0.070	0.069	0.044
250.00	0.125	0.125	0.125	0.100
500.00	0.275	0.267	0.271	0.246
1000.00	0.681	0.687	0.684	0.659
2000.00	1.675	1.713	1.694	1.669



SENSITIVITY

The minimum detectable dose (MDD) of TGF-β1 is typically less than 3.36 pg/ml.
The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

PRECISION

Intra-assay Precision (Precision within an assay)
Three serum-based and buffer-based samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three serum-based and buffer-based samples of known concentration were tested in six separate assays to assess inter-assay precision.

	Intra-assay precision				Inter-assay precision		
Sample	1	2	3		1	2	3
n	20	20	20		6	6	6
Mean (pg/ml)	121.0	319.3	788.0		113.7	313.4	802.6
Standard deviation	6.4	8.1	15.4		6.8	14.2	32.5
CV (%)	5.3	2.5	2.0		6.0	4.5	4.1

RECOVERY

The spike recovery was evaluated by spiking 3 levels of rat TGF-β1 into five health rat serum samples. The un-spiked serum was used as blank in these experiments.
The recovery ranged from 83 % to 117 % with an overall mean recovery of 99 %.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of TGF-β1 in rat serum and diluted with Assay Buffer to produce samples with values within the dynamic range of the assay.

	Average (%)	Range (%)
1:2	111	104 - 115
1:4	108	94 - 117
1:8	102	94 - 110
1:16	91	85 - 99

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant rat TGF-β1. The NIBSC/WHO British Standard for rat TGF-β1 89/514 was evaluated in this kit in May 2011. To convert sample values obtained with the BioOceanELISA TGF-β1 Kit to relative approximate NIBSC units, use the equation below:
NIBSC/WHO (89/514) approximate value (U/ml) = 0.01875 × BioOceanELISA TGF-β1 value (pg/ml)

SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy rats were evaluated for the presence of TGF-β1 in this assay.

Sample Matrix	Number of Samples Evaluated	Range (ng/ml)	Detectable (%)	Mean of Detectable (ng/ml)
Serum	30	28.7 - 99.0	100	57.7

Note: The sample range is non-physiological range. The sample range of healthy rats will difference according to species, sample preparation, and testing personnel, equipment varies. The above information is only reference.

SPECIFICITY

This kit could assay both natural and recombinant rat TGF-β1. A panel of substances listed below were prepared at 1 ng/ml in Assay Buffer to determine cross-reactivity. Preparations of the following substances at 1 ng/ml in a mid-range rrTGF-β1 control to determine interference. No significant cross-reactivity or interference was observed.

Human		Mouse	Rat
IFN-γ	IL-12	IFN-γ	IFN-γ
IL-1β	IL-17A	IL-1β	IL-1β
IL-2	IL-21	IL-4	IL-4
IL-4	IL-22	IL-6	IL-6
IL-5	IL-23	IL-10	IL-10
IL-6	MCP-1	TNF-α	TNF-α
IL-8	TNF-α		
IL-10	VEGF		

PLATE LAYOUT