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Sunlong Medical™ Human TGF-Beta1 Antibody ELISA Kit

Catalog Number: EL0288Hu
Size: 48 Test, 96 Test

Storage: 2-8℃
Validity Period: **Two Years**

Sensitivity:3.36 pg/ml
Assay range:31.25 - 2000 pg/ml

For the **quantitative determination** of human 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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ASSAY PROCEDURE SUMMARY

1. **Prepare all reagents and standards as directed.**

2. **Add 100 µl 2-fold diluted *Standard* in duplicate. Add 100 µl *Assay Buffer (1×)* to Blank well in duplicate.**

3. **Add 50 µl *Assay Buffer (1×)* and 50 µl prediluted sample to the sample well (The dilution refers to the Sample Activation on Page 6).**

4. **Add 50 µl diluted *Detect Antibody* to each well. Step 2, 3 and 4 should be completed within 15 minutes.**

5. **Incubate for 2 hours at RT.**

6. **Aspirate and wash 6 times.**

7. **Add 100 µl *Streptavidin-HRP* to each well.**

8. **Incubate for 45 minutes at RT.**

9. **Aspirate and wash 6 times.**

10. **Add 100 µl *Substrate Solution* to each well. Incubate for 5 - 30 minutes at RT. Protect from light.**

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

12. **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 controls proliferation, differentiation, and other functions in many cell types. TGF-βs act synergistically with TGFA in inducing transformation. 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 (or leukocytes) 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

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human TGF-β1 has been pre-coated onto a microplate. Standard, samples and biotin-linked detect antibody specific for TGF-β1 are pipetted into the wells and TGF-β1 present is bound by the immobilized antibody and detect antibody following incubation. After washing away any unbound substances, streptavidin-HRP is added. After washing, substrate solution is added to the wells and color develops in proportion to the amount of TGF-β1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.
- This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED (96 Test)

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 human TGF-β1.
- **TGF-β1 Standard** (2 vials): Recombinant human TGF-β1 in a buffered protein base with preservatives; lyophilized.
- **TGF-β1 Detect Antibody** (1 vial, 80 µl): Biotin-conjugate anti-human TGF-β1 detect 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
HCl
NaOH</td><td>Up to 1 monthat 2 - 8℃.</td></tr>
<tr><td>Standard</td><td>Up to 1 month at ≤ -20℃ in a manual defrost freezer. Discard after use.</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

- All chemicals should be considered as potentially hazardous.
- We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
- Care should betaken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- The Stop Solution provided with this kit is an acid solution. Wear eyes, hand, face, and clothing protection when using this material.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage and incubation.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- 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.
- In order to avoid microbial contamination or cross- contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the HRP and antibody conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- 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.
- In some cases, an insoluble precipitate of stabilizing protein has been seen in the Standard Diluent. This precipitate does not interfere in anyway with the performance of the test and can thus be ignored. Or remove precipitate by centrifuging at 6,000 ×g for 5 minutes.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution.
- The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
- It is recommended that all samples and standards be assayed in duplicate.
- Take care not to scratch the inner surface of the microwells.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates – Remove particulates by centrifugation at 300 ×g for 10 minutes and assay immediately or aliquotand store samples at ≤ -20℃.

Note: Animal serum used in the preparation of cell culture media may contain high levels of latent TGF-β1. For best results, do not use animal serum for growth of cell culture when assaying for TGF-β1 production. If animal serum is used as a supplement in the media, precautions should be taken to prepare the appropriate control and run the control in the immunoassay to determine the baseline concentration of TGF-β1.

Serum – Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1,000 ×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 ≤ -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 1,000 ×g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 ×g for 10 minutes at 2 - 8℃ is recommended for complete platelet removal. Assay immediately or aliquotand 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 ofTGF-β1, platelet-poor plasma should be collected for measurement. It should be noted that many protocols for Clinical Laboratory Standards (NCCLS), result in incomplete 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.

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 µlAssay Buffer (1×) Plasma: Active 80 µl + 80 µlAssay 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 appropriate dilution factor. Serum: final 40 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.

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 1,600 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.

REAGENT PREPARATION

Bring all reagents and samples to room temperature before use.

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.

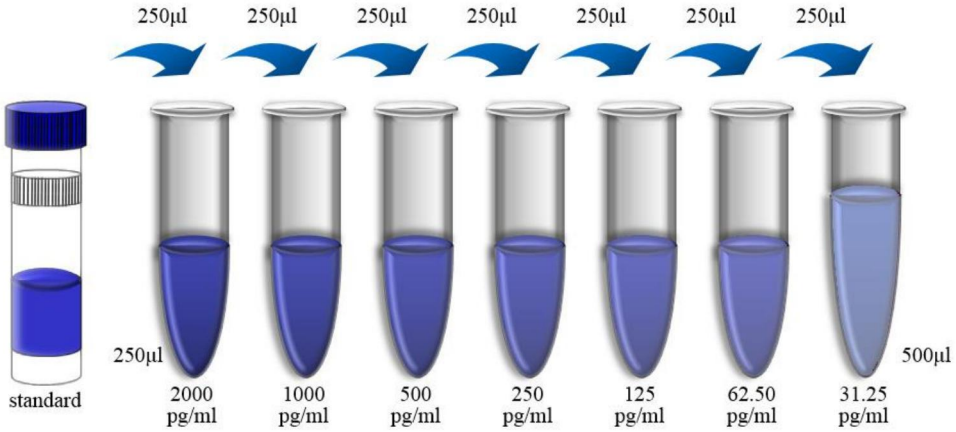
Human TGF-β1 Standard

Reconstitute **Human 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 = 4,000 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 human TGF-β1 standard* (250 µl) with 250 µl of *Assay Buffer (1×)* creates the high standard (2,000 pg/ml). Pipette 250 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 human TGF-β1 standard* (250 µl) with 250 µl of cell culture medium creates the high standard (2,000 pg/ml). Pipette 250 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, andreseal 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 100 µl of 2-fold diluted *Standard* in duplicate. Add 100 µl of *Assay Buffer (1×)* to Blank well in duplicate.
5. Add 50 µl of *Assay Buffer (1×)* and 50 µl prediluted sample to the sample well (The dilution refers to the Sample Activation on Page 6).
6. Add 50 µl of diluted *Detect Antibody* to each well. Ensure reagent addition in step 4, 5 and 6 is uninterrupted and completed within 15 minutes.
7. Cover with an adhesive strip. Incubate at room temperature (18 to 25℃) for 2 hours on a microplate shaker set at 300 rpm.
8. Aspirate each well and wash, repeating the process five times for a total six washes. Wash by filling each well with 300µl *Washing Buffer (1×)*. 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.
9. Add 100 µl of diluted *Streptavidin-HRP* to each well.

10. Cover with a new adhesive strip. Incubate at room temperature (18 to 25℃) for 45 minutes on a microplate shaker set at 300 rpm.
11. Repeat aspiration/wash as in step 8.
12. Add 100 µl of *Substrate Solution* to each well. Incubate for 5 - 30 minutes at room temperature. Protect from light.
13. 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.
14. 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.

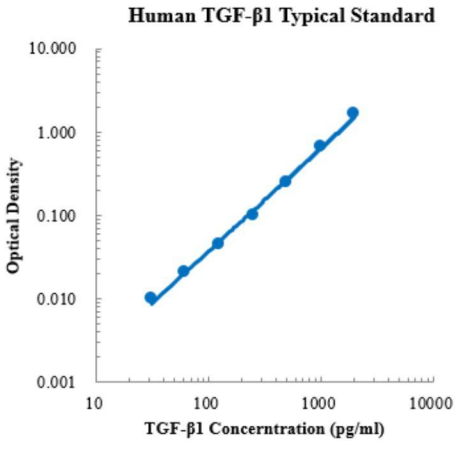
Note: The finally concentration of top standard is 2,000 pg/ml. If instruction in this protocol have been followed samples have been diluted by 1:1 ratio (50 µl prediluted sample + 50 µl Assay Buffer), the concentration read from the standard curve must be multiplied by the dilution factor (×2).

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 human TGF-β1 into five health human 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 human 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 human TGF-β1. The NIBSC/WHO British Standard for human TGF-β1 89/514 was evaluated in this kit in May 2011. To convert sample values obtained with the Human TGF-β1 ELISA Kit to relative approximate NIBSC units, use the equation below:

NIBSC/WHO (89/514) approximate value (U/ml) = 0.01875 × SUNLONG MEDICAL™ Human TGF-β1 value (pg/ml)

SAMPLE VALUES

Serum/Plasma - Thirty samples from apparently healthy volunteers were evaluated for the presence of TGF-β1 in this assay. No medical histories were available for the donors used in this study.

Sample Matrix	Number of Samples Evaluated	Range (ng/ml)	Detectable (%)	Mean of Detectable (ng/ml)
Serum	30	2.1 - 62.2	100	39.3

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

SPECIFICITY

This kit could assay both natural and recombinant human 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 rhTGF-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