SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax: 0086-571-56623318
E-mail: sales@sunlongbiotech.com
www.sunlongbiotech.com

Sunlong Medical™ Human TGF beta 1 One-Step ELISA Kit

Catalog Number: One-EL228Hu
Size: 96 Test

Storage: 2-8℃/-20℃
Validity Period: 2-8℃: 6 months; -20℃: 12 months

Sensitivity: 0.4 pg/mL
Assay range: 31.25 -2000 pg/mL

For the quantitative detection of human TGF beta 1 concentrations in serum, plasma and urine.

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.

TABLE OF CONTENTS

ASSAY PROCEDURE SUMMARY 1

Introduction

Description 2

Principle of the Assay 2

Limitations of the Procedure 2

General Information

Materials Provided 2

Storage 3

Other Supplies Required 3

Precaution 3

Technical Hints 3

Assay Protocol

Sample Collection and Storage 4

Reagent Preparation 4

Assay Procedure 5

Analysis

Calculation of Results 6

Typical Data 6

Sensitivity 7

Precision 7

Recovery 8

Linearity 8

Calibration 8

Sample Values 8

Specificity 8

ASSAY PROCEDURE SUMMARY

1. Preset the layout of the microplate, including control group, standard group and sample group, take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

↓

2. Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

↓

3. Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available).

↓

4. Wash: 1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels. 2) Wash 4 times with 1× Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

↓

5. Add 100 µL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

↓

6. Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

↓

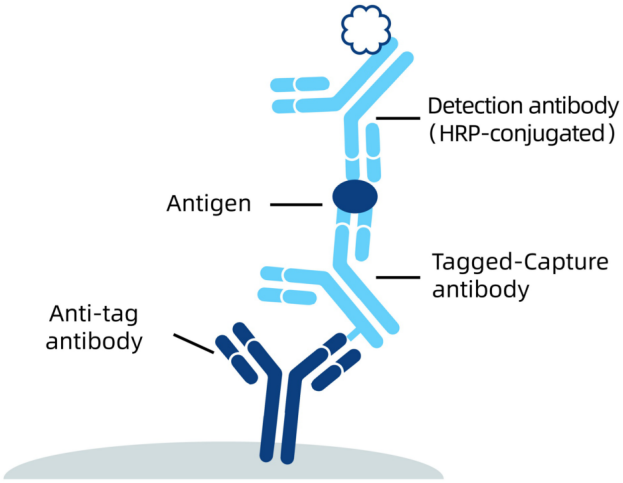
7. Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

DESCRIPTION

TGF-beta is a member of the transforming growth factor beta (TGFB) family of cytokines, which are multifunctional peptides that regulate proliferation, differentiation, adhesion, migration, and other functions in many cell types. TGF-beta is produced by a number of cell types including regulatory T cells, fibroblasts, epithelial cells, and endothelial cells. TGF-beta acts synergistically with TGFA in inducing transformation. It also acts as a negative autocrine growth factor. TGF-beta plays an important role in bone remodeling as it is a potent stimulator of osteoblastic bone formation, causing chemotaxis, proliferation and differentiation in committed osteoblasts. TGF-beta appears to promote late stage progression and metastasis in some cancers.

PRINCIPLE OF THE ASSAY

An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the solution yellow. The color intensity is proportional to the quantity of bound protein, which is measurable at 450 nm with the correction wavelength set at 630 nm.



LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use expired kit or reagents.
- Do not mix or substitute reagents or materials from other kit lots or other sources.
- If concentration of assayed factor in samples is higher than the highest standard, dilute the samples with the appropriate Sample Diluent and reanalyze.
- 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℃.

- **Microplate:** 96 well microplate precoated an anti-tag antibody (8 well × 12 strips) (1 plate)
- **Protein standard:** 4000 pg/bottle; lyophilized (2 bottles)
- **Capture antibody (100×):** 60 uL/vial* (1 vial)

- **Detection antibody, HRP-Conjugated (100×):** 60 uL/vial* (1 vial)
- **Sample Diluent PT 4B1:** 30 mL/bottle (1 bottle)
- **Detection Diluent:** 15 mL/bottle (1 bottle)
- **Wash Buffer Concentrate (20×):** 30 mL/bottle (1 bottle)
- **Tetramethylbenzidine Substrate (TMB):** 12 mL/bottle (1 bottle)
- **Stop Solution:** 12 mL/bottle (1 bottle)
- **Activator reagent I (1M HCl):** 4 mL/bottle (1 bottle)
- **Activator reagent II (1.2M NaOH/0.5 M HEPES):** 4 mL/bottle (1 bottle)
- Plate Cover Seals (4 pieces)

STORAGE

Store kit reagents between 2 and 8°C. Immediately after use remaining reagents should be returned to cold storage (2 to 8°C). Expiration date of the kit and reagents is stated on the labels.

Expiration date of the kit components can only be guaranteed if the components are stored properly.

	Condition
Unopened kit	Store at 2 - 8°C for 6 months or -20°C for 12 months. (See expiration date on the label.)
Opened kit	All reagents stored at 2 - 8°C for 7 days. Please use a new standard for each assay.

OTHER SUPPLIES REQUIRED

- A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.
- Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.
- Plate washer: automated or manual.
- Absorbent paper towels.
- Glass or plastic tubes to prepare standard and sample dilutions.
- Beakers and graduated cylinders.
- Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.
- Microplate thermostatic shaker.

PRECAUTION

- Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
- Do not use the kit after the expiration date.
- Do not mix or substitute reagents or materials from other kit lots or other sources.
- Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

TECHNICAL HINTS

- Bring all reagents to room temperature before use.
- 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 second 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.

SAMPLE COLLECTION AND STORAGE

Serum

Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20℃. Avoid repeated freeze-thaw cycles.

Plasma

Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20℃. Avoid repeated freeze-thaw cycles.

Urine

Collect urine samples and centrifuge for 20 minutes at 1000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents and samples to room temperature before use. If crystals have formed in the Wash Buffer Concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.

Wash Buffer (1X)

If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

Antibody Cocktail (1X)

Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 uL 100X capture antibody + 50 uL 100X Detection Antibody, HRP-conjugated + 4,900 uL Detection Diluent. Mix gently but thoroughly.

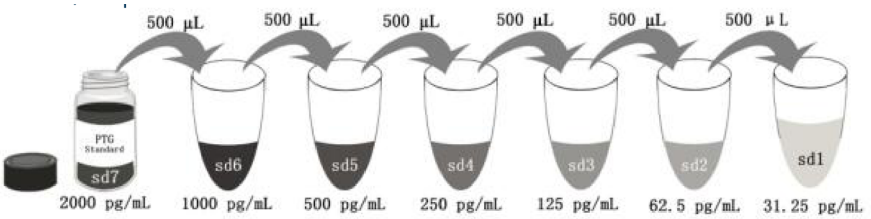
Sample Dilution

Different samples should be diluted with corresponding Sample Diluent. The minimum required dilution factor was 1:2; consequently, no more than 25 uL of undiluted sample was added per well. Samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement. To activate latent TGF beta 1 to the immunoreactive form, prepare the following solutionsfor acid activation and neutralization. The solutions may be stored in polypropylene bottles at room temperature for up to one month. 1 N HCl (100 mL) To 91.67 mL of deionized water, slowly add 8.33 mL of 12 N HCl. Mix well. 1.2 N NaOH/ 1 0.5 M HEPES (100 mL) To 75 mL of deionized water, slowly add 12 mL of 10 N NaOH. Mix well. Add 11.9 g of HEPES. Mix well. Bring final volume to 100 mL with deionized water. For each new lot of acidifcation and neutralization reagents, measure the pH of several representative samples after neutralization to ensure that it is within pH 7.2-7.6. Adjust the volume and corresponding dilution factor of the neutralization reagent as needed. Recommended Dilution for different sample types: 1:160 or 1:320 is recommended for human serum and plasma; 1:2.8 or 1:5.6 is recommended for urine. Note: The concentration reads must be multiplied by the dilution factors and recommended dilution. Human serum and plasma samples were first activated with an

activator (equivalent to a 2-fold dilution), then diluted 80fold with sample diluent PT 4B1. Using a total volume of 600 uL as an example, the recommended dilution protocol is as follows: take 7.5 uL of the activated sample (already 2-fold diluted) and add 592.5 uL of the sample diluent PT 4B1 (final dilution factor of sample is 160). Urine samples were first activated with an activator (equivalent to a 1.4-fold dilution), then diluted 2-fold with sample diluent PT 4B1. Using a total volume of 600 uL as an example, the recommended dilution protocol is as follows: take 300 uL of the activated sample (already 1.4-fold diluted) and add 300 uL of the sample diluent PT 4B1 (final dilution factor of sample is 2.8).

Standard Serial Dilution

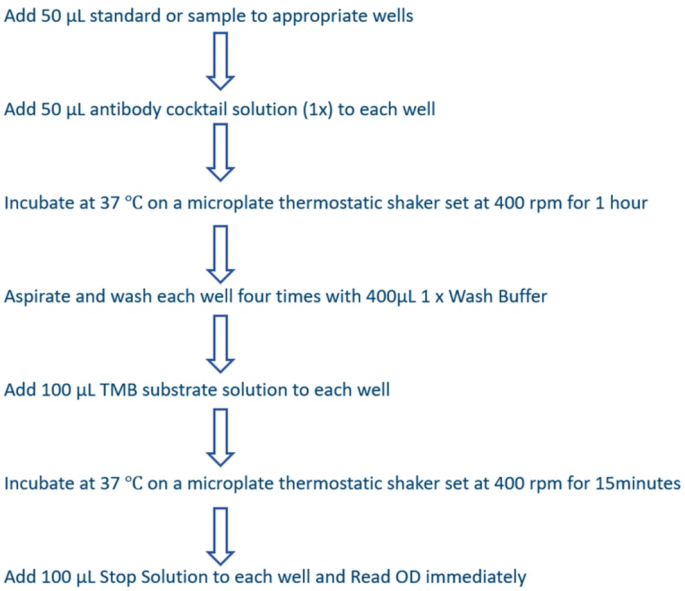
Add 2 mL Sample Diluent PT 4B1 in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

ASSAY PROCEDURE

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated antibody can be used immediately). 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.



1. Preset the layout of the microplate, including control group, standard group and sample group, take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

2. Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

3. Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37℃ on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37℃ for 2 hours is recommended if thermostatic shaker is not available).

4. Wash: 1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels. 2) Wash 4 times with 1× Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

5. Add 100 µL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

6. Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

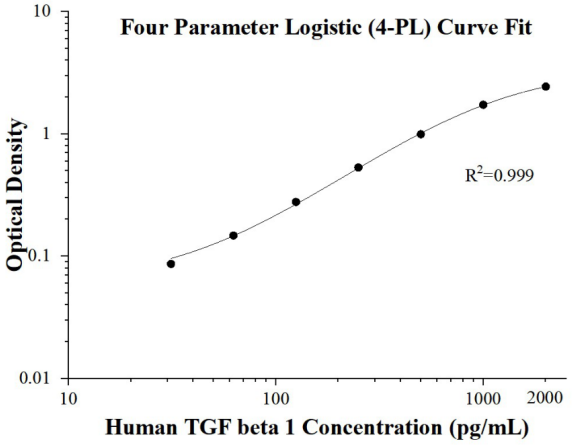
7. Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

CALCULATION OF RESULTS

Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, four-parameter logistic curvefit (4-PL) analysis is recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.0282 0.0298	0.0290	-
31.25	0.1122 0.1188	0.1155	0.0865
62.5	0.1775 0.176	0.1768	0.1478
125	0.2921 0.3217	0.3069	0.2779
250	0.5433 0.5771	0.5602	0.5312
500	1.0394 1.0046	1.0220	0.9930
1000	1.7701 1.7538	1.7620	1.7330
2000	2.4636 2.4651	2.4644	2.4354

SENSITIVITY

The minimum detectable dose of human TGF beta 1 is 0.4 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

PRECISION

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision. Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision

Sample	n	Mean (pg/mL)	SD	CV%
1	8	1,030.1	22.0	2.1
2	8	258.2	6.4	2.5
3	8	129.0	3.8	2.9

Inter-assay Precision

Sample	n	Mean (pg/mL)	SD	CV%
1	16	1,036.5	20.7	2.0
2	16	260.8	11.9	4.6
3	16	127.0	4.6	3.6

RECOVERY

The recovery of human TGF-beta1 spiked to three different levels throughout the range of the assay in in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:640	97	95-99
	1:1,280	96	94-98
Urine	1:2.8	79	74-85

LINEARITY

To assess the linearity of the assay, urinet samples were spiked with high concentrations of human TGF beta 1 and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. Human serum samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The activated human serum was initially diluted 1:40.)

		Human serum	Urine
1:2	Average% of Expected	100	97
	Range (%)	-	91-103
1:4	Average% of Expected	108	94
	Range (%)	107-109	91-96
1:8	Average% of Expected	118	103
	Range (%)	114-121	102-105
1:16	Average% of Expected	124	107
	Range (%)	120-128	105-108

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant **human TGF beta 1** produced at Sunlong Medical™.

SAMPLE VALUES

Human serum/Urine - Human serum and urine samples were evaluated for the presence of human TGF beta 1 in this assay. ND*=Non-detectable

Sample Type	Mean (ng/mL)	Range (ng/mL)
Human serum (n=16)	67.9	7.3-120.8

Sample Type	Mean (pg/mL)	%Detectable	Range (pg/mL)
Urine (n=8)	48.3	37.5	ND-59.7

SPECIFICITY

This assay recognizes natural and recombinant human TGF beta 1. The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference. Recombinant human: Recombinant mouse: Recombinant rat: BMP-3 TGF-β RI TGF beta 1 GDF-15 TGF-β RII BMP-5 BMPR-IA BMP-10 BMP-3 BMP-7 BMPR-IB Agrin TGF-β RI TGF-α BMPR-IB Activin A BMP8A BMP6 BMP8B BMP-2 BMP-4 TGF-β1 BMPR-1A GDF-11 A sample containing 50 ng/mL of the recombinant mouse TGF beta 1 reads as 50 pg/mL (0.10% cross-reactivity).

PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												