

# Human TGF- $\beta$ 1 ELISA Kit

For the quantitative determination of human transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) concentrations in serum, plasma, cell culture supernatant, and other biological fluids

Catalogue Number: EL10029

*96 tests*

FOR LABORATORY RESEARCH USE ONLY  
NOT FOR USE IN DIAGNOSTIC PROCEDURES



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## INTENDED USE

This Human TGF- $\beta$ 1 ELISA kit is to be used for the *in vitro* quantitative determination of human transforming growth factor beta 1 (TGF- $\beta$ 1) concentrations in serum, plasma, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

## INTRODUCTION

Transforming growth factor beta (TGF- $\beta$ ) is a stable, multifunctional polypeptide growth factor. While specific receptors for this protein have been found on almost all mammalian cells examined, the effect of the molecule varies depending on the cell type and growth conditions. Generally, TGF- $\beta$  is stimulatory for cells of mesenchymal origin and inhibitory of cells of epithelial or neuroectodermal origin. The originally described form of TGF- $\beta$ , now designated TGF- $\beta$ 1, is only one member of a family of regulatory proteins consisting of a number of factors distantly related to TGF- $\beta$ 1 (30-40% sequence identity), including the activins, inhibins, and bone morphogenetic proteins (BMPs), and a number of more closely related proteins (70-80% sequence identity) designated TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, TGF- $\beta$ 4, and TGF- $\beta$ 5.<sup>1-4</sup>

Most of the currently published reports on the activities of TGF- $\beta$  have dealt only with TGF- $\beta$ 1. In general, TGF- $\beta$ 1, TGF- $\beta$ 2 and TGF- $\beta$ 3 appear to be functionally equivalent in biological activity *in vitro*, although there seem to be differences in potency on different cell types.<sup>5-8</sup> TGF- $\beta$ 1 has been found in the highest concentration in human platelets and mammalian bone, but it is produced in smaller amounts by many cells.<sup>1,2,9</sup> TGF- $\beta$  has a wide range of biological activities. With only a few exceptions, all cells have surface receptors for, and respond to TGF- $\beta$ .<sup>1-4</sup> TGF- $\beta$  is an important modulator of the growth, differentiation, and activities of a number of the different types of cells involved in both cellular and humoral immune responses. TGF- $\beta$  is a potent suppressor of the proliferation of T and B lymphocytes, thymocytes, and some immature hematopoietic cell populations.<sup>1-4, 10</sup> TGF- $\beta$ 1 enhances the deposition of extracellular matrix through promotion of synthesis and inhibition of degradation. TGF- $\beta$ 1 is immunosuppressive through a variety of mechanisms.<sup>11</sup> The specific action of TGF- $\beta$ 1 on a particular cell depends on the exact circumstances of that cell's environment.

The most commonly used bioassays for TGF- $\beta$ 1 are based on its anti-proliferative effects of various cell lines, e.g., mink lung epithelial cells (CCL64)<sup>12</sup> or HT-2 murine helper T cells.<sup>13</sup> These assays require two days to complete and lack the specificity of distinguish between the various isoforms of TGF- $\beta$ 1. Human TGF- $\beta$ 1 ELISA Kit is a 3 hours solid phase ELISA designed to measure TGF- $\beta$ 1 in cell culture supernatant, serum and plasma. Results obtained using natural TGF- $\beta$ 1 showed linear curves that were parallel to the standard curves obtained using the CHO-derived kit standards. Since TGF- $\beta$ 1 is not

glycosylated, it is virtually certain that the Human TGF- $\beta$ 1 ELISA Kit will provide accurate quantitation for both recombinant and natural TGF- $\beta$ .

*This TGF- $\beta$ 1 ELISA is a 3.5 hour solid phase immunoassay readily applicable to measure TGF- $\beta$ 1 levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 2000 pg/mL. It showed no cross reactivity with various other cytokines. This TGF- $\beta$ 1 ELISA is expected to be effectively used for further investigations into the relationship between TGF- $\beta$ 1 and various diseases.*

## **PRINCIPLE OF THE ASSAY**

This TGF- $\beta$ 1 enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to TGF- $\beta$ 1. Standards or samples are then added to the appropriate microtiter plate wells and incubated. After washing, biotin-conjugated polyclonal antibody specific to TGF- $\beta$ 1 is added and incubated. TGF- $\beta$ 1 if present, will bind and become immobilized by the antibody pre-coated on the wells and then be “sandwiched” by biotin conjugate. The microtiter plate wells are thoroughly washed to remove unbound TGF- $\beta$ 1 and other components of the sample. In order to quantitatively determine the amount of TGF- $\beta$ 1 present in the sample, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Avidin is a tetramer containing four identical subunits that each has a high affinity-binding site for biotin. The wells are thoroughly washed to remove all unbound HRP-conjugated Avidin and a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain TGF- $\beta$ 1, biotin-conjugated antibody, and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm  $\pm$  2 nm.

In order to measure the concentration of TGF- $\beta$ 1 in the samples, this kit includes two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing.) According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D) versus TGF- $\beta$ 1 concentration (pg/mL). The concentration of TGF- $\beta$ 1 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

## **LIMITATIONS OF THE PROCEDURE**

- FOR LABORATORY RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- As manufacturers we take great care to ensure that our products are suitable for use with all validated sample types, as designated in the product insert. However, it is possible that in some cases, high levels of interfering factors may cause unusual results.
- The kit should not be used beyond the expiration date on the kit label.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Soluble receptors or other binding proteins present in biological samples do not necessarily interfere with the measurement of ligands in samples. However, until the factors have been tested, the possibility of interference cannot be excluded.

## REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

		<b>96 tests</b>
1.	<b>TGF-β1 MICROTITER PLATE</b> (Part EL29-1) _____ Pre-coated with anti-human TGF-β1 monoclonal antibody.	<b>96 wells</b>
2.	<b>BIOTIN CONJUGATE</b> (EL29-2) _____ Anti-human TGF-β1 polyclonal antibody conjugated to Biotin.	<b>6 mL</b>
3.	<b>AVIDIN HRP CONJUGATE</b> (Part EL29-3) _____ Avidin conjugated to horseradish peroxidase.	<b>12 mL</b>
4.	<b>TGF-β1 STANDARD</b> (Part EL29-4) _____ Recombinant human TGF-β1 (4000 pg/vial) in a buffered protein base with preservative, lyophilized.	<b>2 vials</b>
5.	<b>TGF-β1 CALIBRATOR DILUENT I</b> (Part EL29-5) _____ Animal protein with buffer and preservative. For TGF-β1 serum/plasma testing.	<b>30 mL</b>
6.	<b>TGF-β1 CALIBRATOR DILUENT II</b> (Part EL29-6) _____ Cell culture medium with animal protein and preservative. <i>For TGF-β1 cell culture supernatant testing.</i>	<b>25 mL</b>
7.	<b>WASH BUFFER (20X)</b> (Part 30005) _____ 20-fold concentrated solution of buffered surfactant.	<b>60 mL</b>
8.	<b>SUBSTRATE A</b> (Part EL29-7) _____ Buffered solution with H <sub>2</sub> O <sub>2</sub>	<b>10 mL</b>
9.	<b>SUBSTRATE B</b> (Part 30007) _____ Buffered solution with TMB.	<b>10 mL</b>
10.	<b>STOP SOLUTION</b> (Part 30008) _____ 2N Sulphuric Acid (H <sub>2</sub> SO <sub>4</sub> ). Caution: Caustic Material!	<b>14 mL</b>

## **MATERIALS REQUIRED BUT NOT SUPPLIED**

1. Single or multi-channel precision pipettes with disposable tips: 10-100 $\mu$ L and 50-200 $\mu$ L for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm  $\pm$  2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

## **ADDITIONAL REAGENTS REQUIRED FOR SAMPLE ACTIVATION:**

- HEPES, free acid (Reagent Grade, M.W.238.8)
- Hydrochloric acid (A.C.S. Grade, 12N)
- Sodium hydroxide (A.C.S Grade, 10N)

## **PRECAUTIONS**

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

9. All samples should be disposed of in a manner that will inactivate human viruses.  
Solid Wastes: Autoclave for 60 minutes at 121°C.  
Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the virus before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

## **SAMPLE COLLECTION, HANDLING AND STORAGE**

- a) **Cell Culture Supernatant:** Collect cell culture supernatant, Centrifuge to remove any visible pellets. Assay can be immediately conducted or samples can be aliquoted and store at  $\leq -70^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles. Special caution: The supernatant may contain a certain level of latent TGF- $\beta$ 1 if bovine serum is added as a supplement to the media. To achieve best results, avoid using such media or if it is inevitable create an appropriate approach to determine the base line level of TGF- $\beta$ 1.
- b) **Serum:** Use a serum separator tube (SST) and allow samples to clot for one hour at room temperature. For complete release of TGF- $\beta$ 1, incubate overnight at 2-8°C before centrifugation. Centrifuge for 10 minutes at 1000 x g (4°C). Remove serum and assay (see activation procedure) immediately or aliquot and store at  $\leq -70^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.
- c) **Plasma:** Collect plasma on ice using EDTA or CPDA-1 as an anticoagulant. Centrifuge at 1000 x g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 x g for 10 minutes at 2-8°C is recommended for complete platelet removal. Assay (see activation procedure) immediately or aliquot and store samples at  $\leq -70^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

TGF- $\beta$ 1 is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of TGF- $\beta$ 1, platelet-poor plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the National Committee for Clinical Laboratory Standards (NCCLS), result in incomplete removal of platelets from Blood. This will cause variable and irreproducible results for assays of factors contained in platelets 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, it is recommended that markers for platelet degranulation be determined in samples containing elevated TGF- $\beta$ 1 levels.



## ACTIVATION REAGENT PREPARATION

To activate latent TGF- $\beta$ 1 to the immuno-reactive form, prepare the following solutions for acid activation and neutralization. The solutions may be stored in polypropylene bottles at room temperature for up to one month. If any precipitate forms gently heat the solution to 37°C while mixing, cool to room temperature before use. *Caution: Wear protective clothing and safety glasses during preparation or use of these reagents.*

### Reagents to activate cell culture supernatants, plasma and serum.

Reagent	Preparation
1N HCl (100mL)	Slowly add 8.33mL 12N HCL to 91.67mL deionized water while stirring.
1.2 N NaOH/0.5M HEPES (100 mL)	Slowly add 12mL 10N NaOH to 75 mL deionized water, mix well. Add 11.9g HEPES to the above solution. Stir to dissolve completely. Add deionized water to have a final volume of 100 ml.

## TGF- $\beta$ 1 SAMPLE ACTIVATION PROCEDURE

To activate latent TGF- $\beta$ 1 to immuno-reactive TGF- $\beta$ 1 detectable by Human TGF- $\beta$ 1 ELISA Kit, follow the activation procedure outlined below. Assay samples after neutralization (pH 7.2 - 7.6) and use polyporpylene test tubes.

*Notes: Do not activate the kit standards. The kit standards contain active rhTGF- $\beta$ 1. If samples generate values higher than the highest standard, dilute the samples after activation with the appropriate Calibrator Diluent and repeat the assay.*

Cell Culture Supernatant	Serum/Plasma
To 0.5mL cell culture supernatant, add 0.1mL 1N HCL.	To 0.1mL serum, add 0.05mL 1N HCL.
Mix well	Mix well
Incubate 10 minutes at room temperature.	Incubate 10 minutes at room temperature.
Neutralize the acidified sample by adding 0.1mL 1.2N NaOH/0.5M HEPES.	Neutralize the acidified sample by adding 0.05mL 1.2N NaOH/0.5M HEPES.
Mix well	Mix well
Assay immediately	Prior to the assay, dilute the activated serum/plasma samples 15-100 fold with Calibrator Diluent I. *
The concentration read off the standard curve must be multiplied by the dilution factor, 1.4.	The concentration read off the standard curve must be multiplied by the dilution factor 30(15 fold) or dilution factor 200 (100 fold).

\*Note: Serum samples contain higher level of TGF- $\beta$ 1 than plasma samples in general. It is suggested to refer to the expected sample value to dilute serum/plasma samples. If diluted samples are not within assay range, adjust the dilution scale.

For each new lot of acidification 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.

### Cell Culture Supernatants Note

Significant levels of latent TGF- $\beta$ 1 are found in bovine, porcine, equine, and caprine sera which can be as high as 16ng/mL after activation (ref. 12, 13). The background level of TGF- $\beta$ 1 in control medium can be determined and subtracted from samples of such medium. On the other hand, the background level of TGF- $\beta$ 1 in medium can be lowered using the following procedure prior to assaying (ref. 12 with modifications). After growth to confluence in medium containing 10 %serum, the medium is changed to serum-free medium supplemented with 200 $\mu$ g/mL crystalline BSA with four changes of medium over 12-24 hours. Cells are then switched to medium alone or medium containing 200 $\mu$ g/mL crystalline BSA. Specific additives may be required for particular cell lines for maintenance. After 24 hours, the serum-free conditioned medium is clarified by centrifugation and samples are stored at  $\leq$ -70°C. Optionally, protease inhibitors such as aprotinin, leupeptin, pepstatin A, and PMSF can be added before freezing. Thawed or fresh samples of serum-free or serum-containing conditioned medium are processed further as described above. If bovine serum added as a supplement to conditioned media exceeds 5%, further dilute the activated sample at least 2-fold using Calibrator Diluent II. The dilution as a result of the sample activation procedure (1.4 fold) should be taken into consideration in the final calculation of the concentration of TGF- $\beta$ 1 in culture media samples.

### PREPARATION OF REAGENTS

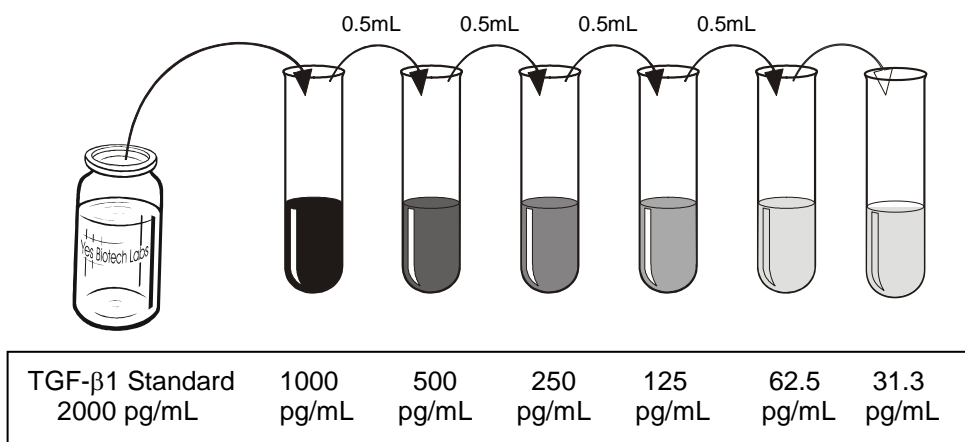
Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

### 3. **TGF- $\beta$ 1 Standard:**

- Two vials of Standards are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute the TGF- $\beta$ 1 Standard with either 2.0mL of Calibrator Diluent I (For serum/plasma testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 2,000pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The TGF- $\beta$ 1 standard stock solution must be stored frozen (-20°C) immediately after use so that it can last for up to 30 days. Avoid freeze-thaw cycles. Aliquot if repeated use is expected.
- Use the above stock solution to produce a serial 2-fold dilution series within the range of this assay (31.3pg/mL to 2,000pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted TGF- $\beta$ 1 Standard will serve as the **high standard (2000pg/mL)** and the Calibrator Diluent will serve as the zero standard (0pg/mL).



## ASSAY PROCEDURE

1. Prepare Wash Buffer and TGF- $\beta$ 1 Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards and Samples to the Microtiter Plate.*

Wells				Contents				Wells				Contents			
1A, 1B		Standard 1		0 pg/mL		(S1)		2A, 2B		Standard 5		250 pg/mL		(S5)	
1C, 1D		Standard 2		31.3 pg/mL		(S2)		2C, 2D		Standard 6		500 pg/mL		(S6)	
1E, 1F		Standard 3		62.5 pg/mL		(S3)		2E, 2F		Standard 7		1000 pg/mL		(S7)	
1G, 1H		Standard 4		125 pg/mL		(S4)		2G,2H		Standard 8		2000 pg/mL		(S8)	
3A-12H		Samples													
	1	2	3	4	5	6	7	8	9	10	11	12			
A	S1	S5	1	5	9	13	17	21	25	29	33	37			
B	S1	S5	1	5	9	13	17	21	25	29	33	37			
C	S2	S6	2	6	10	14	18	22	26	30	34	38			
D	S2	S6	2	6	10	14	18	22	26	30	34	38			
E	S3	S7	3	7	11	15	19	23	27	31	35	39			
F	S3	S7	3	7	11	15	19	23	27	31	35	39			
G	S4	S8	4	8	12	16	20	24	28	32	36	40			
H	S4	S8	4	8	12	16	20	24	28	32	36	40			

2. Add 25 $\mu$ L of Standard or Sample to the appropriate well of the antibody pre-coated Microtiter Plate and incubate for 1 hour at room temperature.
3. Wash the Microtiter Plate using one of the methods indicated below:

**Manual Washing:** Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for a **total of two washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note:* Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

**Automated Washing:** Aspirate all wells, then wash plates **two times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350  $\mu$ L/well/wash (range: 350-400  $\mu$ L). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

4. Add 50 $\mu$ L TGF- $\beta$ 1 Biotin conjugate to each wells. Cover and incubate for 1 hour at room temperature.

5. Wash plates **FIVE** times as described in Step 3.
6. Dispense 100 $\mu$ l of Avidin HRP Conjugate to each well Mix well. Cover and incubate for 1 hour at room temperature.
7. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).
8. Wash plates **FIVE** times as described in Step 3.
9. Add 100 $\mu$ L Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.
10. Add 100 $\mu$ L Stop Solution to each well. Mix well.
11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader set within 30 minutes.

## **CALCULATION OF RESULTS**

The standard curve is used to determine the amount of TGF- $\beta$ 1 in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding TGF- $\beta$ 1 concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL) or (S1) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of TGF- $\beta$ 1 in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding TGF- $\beta$ 1 concentration. Because samples have been diluted in the activation step, the measured concentrations must be multiplied by the dilution factor (cell culture supernate multiplied dilution factor 1.4; serum multiplied dilution factor 30)
3. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.

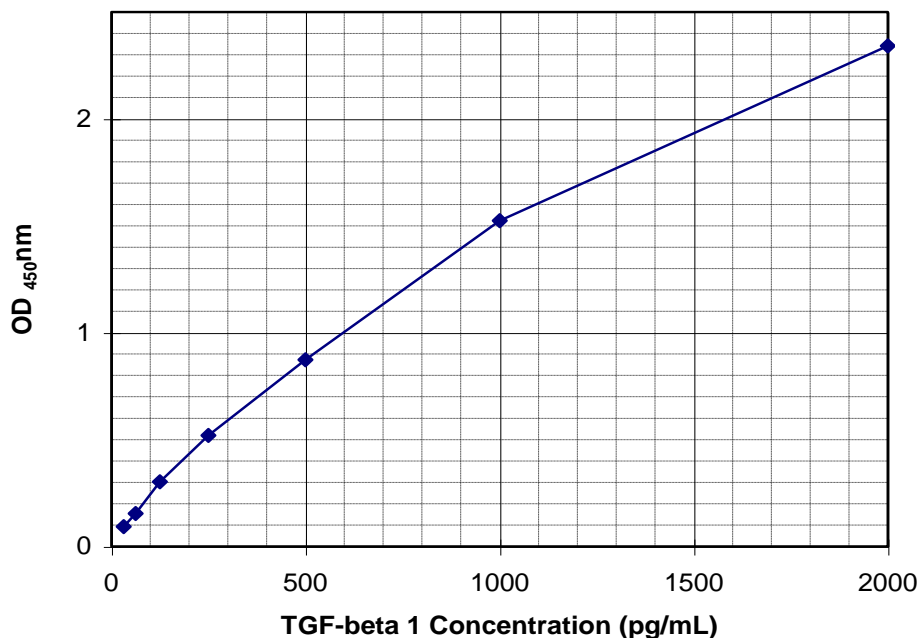
## TYPICAL DATA

Results of a typical standard run of a TGF- $\beta$ 1 ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain own standard curve

### EXAMPLE ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

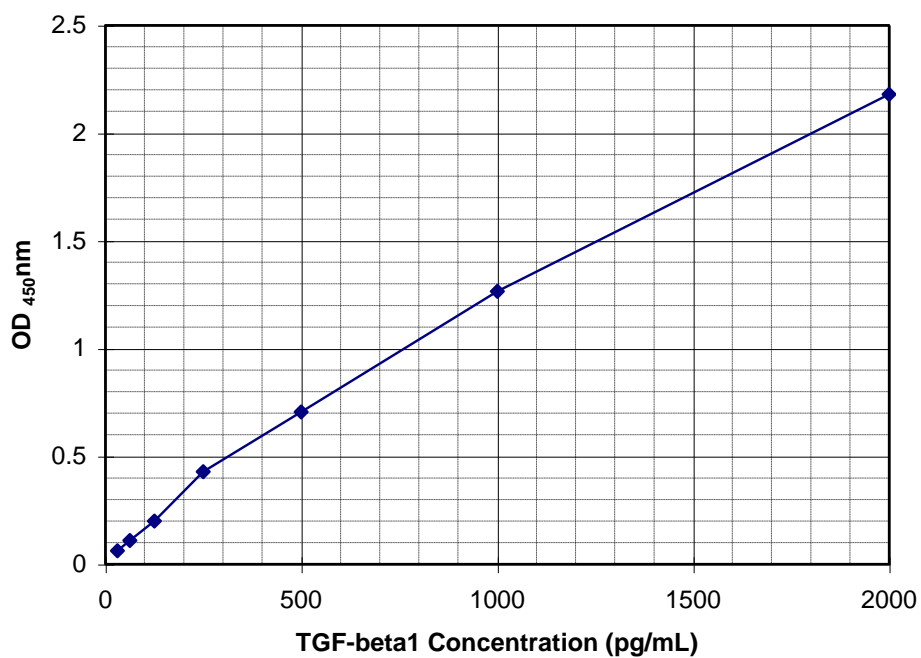
Standard (pg/mL)	Mean	Zero Standard Subtracted (Std.)-(S1)
0	0.0570	0
31.25	0.1500	0.0930
62.5	0.2115	0.1545
125	0.3600	0.3030
250	0.5760	0.5190
500	0.9305	0.8735
1000	1.5810	1.5240
2000	2.3990	2.3420



### **EXAMPLE TWO**

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	Mean	Zero Standard Subtracted (Std.) -(S1)
0	0.052	0
31.25	0.114	0.062
62.5	0.161	0.109
125	0.251	0.199
250	0.481	0.429
500	0.758	0.706
1000	1.317	1.265
2000	2.232	2.180



## PERFORMANCE CHARACTERISTICS

### 1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration were assayed by using 20 replicates in 1 assay.

	<b>Calibrator Diluent I assay</b>			<b>Calibrator Diluent II assay</b>		
<b>Sample</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>1</b>	<b>2</b>	<b>3</b>
N	20	20	20	20	20	20
Mean (pg/mL)	88.0	514	1113	87.8	499	1189
Standard Deviation (pg/mL)	6.4	25.0	41.7	6.6	26.1	68.3
Coefficient of Variation (%)	7.3	4.9	3.7	7.5	5.2	5.7

### 2. INTER-ASSAY PRECISION

To determine between-run precision, three different samples of known concentration were assayed by using replicates on 20 different assays.

	<b>Calibrator Diluent I assay</b>			<b>Calibrator Diluent II assay</b>		
<b>Sample</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>1</b>	<b>2</b>	<b>3</b>
N	20	20	20	20	20	20
Mean (pg/mL)	103	533	1184	92.0	531	1217
Standard Deviation (pg/mL)	12.0	41.0	83.7	5.8	17.1	55.2
Coefficient of Variation (%)	11.7	7.7	7.1	6.3	3.2	4.5

### 3. RECOVERY

The recovery of TGF- $\beta$ 1 spiked to levels throughout the range of the assay followed by activation in various matrices was evaluated.

<b>Sample Type</b>	<b>Average % Recovery</b>	<b>Range</b>
Cell culture media	98	80-116%
Serum	97	84-120%
EDTA plasma (platelet-poor)	108	89-123%

### 4. SENSITIVITY

The minimum detectable dose of TGF- $\beta$ 1 was determined by adding two standard deviations to the mean optical density value of 20 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose using a standard curve generated with Calibrator Diluent I is 9.0 pg/mL and using Calibrator Diluent II is 9.0 pg/mL.

### 5. SPECIFICITY

This sandwich ELISA recognizes both natural and recombinant human TGF- $\beta$ 1. The factors listed below were prepared at 50ng/mL in Calibrator Diluent I and Calibrator Diluent II and assayed for cross-reactivity. No significant cross-reactivity or interference was observed.



Factors related to or associated with TGF- $\beta$ 1:

rhTGF- $\alpha$ , rh TGF- $\beta$  aRIII, rh TGF- $\beta$  aRII, pTGF- $\beta$ <sub>2</sub>

Other factors:

Recombinant Human				Recombinant Mouse	
IL-1 $\alpha$	ANG	IGF-I	SLP1	IL-1 $\alpha$	bFGF acidic
IL-1 $\beta$	CNTF	LIF	TNF- $\alpha$	IL-1 $\beta$	bFGF basic
IL-1 ra	$\beta$ -ECGF	M-CSF	TNF- $\beta$	IL-3	mEGF
IL-2	EGF	MCP-1	sTNF RI	IL-4	
IL-3	EPO	MIC-1 $\alpha$	sTNF RII	IL-5	
IL-4	FGF-basic	MIP-1 $\beta$	VEGF	IL-7	
IL-5	FGF-acidic	$\beta$ -NGF		IL-9	
IL-6	FGF-5	OSM		IL-10	
IL-6 sR	FGF-6	PDGF-AA		EGF	
IL-7	G-CSF	PDGF-AB		GM-CSF	
IL-8	GRO- $\alpha$	PDGF-BB		LIF	
IL-9	HB-EGF	PTN		MIP-1 $\beta$	
IL-10	HGF	PANTES		SCF	
IL-11	IFN- $\gamma$	SCF		TNF- $\alpha$	

Some cross-reactivity and interference was observed with the following:

Cross-reactivity:

Factor	Concentration Tested (pg/mL)	Observed Value (pg/mL)	% Cross-reactivity
rhLatent TGF- $\beta$ 1	10,000	1.244	12.4
rh TGF- $\beta$ 1,2	10,000	671	6.7
rh TGF- $\beta$ 2	50,000	41.9	0.08
rh TGF- $\beta$ 3	50,000	58.4	0.12
rh TGF- $\beta$ 5	50,000	962	1.9

Interference: TGF- $\beta$ 1-related factors added to a mid-level TGF- $\beta$ 1 control were evaluated.

<b>Factor</b>	<b>Concentration Tested ((pg/mL)</b>	<b>TGF-β1 Control (pg/mL)</b>	<b>Observed Value (pg/mL)</b>
rhLatent TGF-β1	10,000	259	953
rh TGF-β1,2	10,000	259	706
rh TGF-β2	50,000	216	304
rh TGF-β3	50,000	260	215
rh TGF-β5	50,000	216	684

#### 6. CALIBRATION

This immunoassay is calibrated against NIBSC Standard (Reference preparation) Code No. 89/514.

#### 7. SAMPLE VALUES

Serum/Platelet-poor Plasma - Serum and plasma samples were activated and evaluated for the presence of TGF-β1 in the assay.

<b>Sample Type</b>	<b>Average (ng/mL)</b>	<b>Range (ng/mL)</b>
Serum (n=40)	48.6	34.7 - 63.9
EDTA " Platelet Poor" plasma (n=10)	2.26	1.58 – 3.24
CPDA-1 plasma	3.31	2.25 – 5.37

## REFERENCES:

1. Spom, M. B. et al. (1987) J. Cell Biol. 105: 1039.
2. Spom, M.B and A. B. Roberts. (1990) "The Transforming Growth Factor- $\beta$ s " in Peptide Growth Factors and Their Receptors I. Spom, M.B. and A. B. Roberts, eds., Springer-Verlag, New York, p.419.
3. Spom, M. B.and A. B. Roberts (1992) J. Cell Biol.119: 1017.
4. Spom, M. B.and A. B. Roberts. (1993) Growth Factors 8: 1.
5. Graycar, J. L. et al. (1990) Mol. Endocrinol. 3: 1977.
6. Ridley, A. J. et al. (1989) J. Cell Biol.109: 3419.
7. ten Dijke, P. et al. (1990) Mol. Cell. Biol. 10: 4473.
8. Cheifetz, S. et al. (1990) J. Biol. Chem. 265: 20533.
9. Cheifetz, S. et al. (1987) Cell 48: 409.
10. Hooper, W.C. (1991) Leuk. Res, 15: 179.
11. Lawrence, D.A. (1996) Eur.Cytokine Netw. 7:363.
12. Spom, M. B. and A. B. Roberts (1990) "The Transforming Growth Factor- $\beta$ s" In Peptide Growth Factors and Their Receptors I, Spom, M. B. and A. B. Roberts eds., Springer-Verlag, NEW York, p419.
13. Tsang, M. et al. (1990) Lymphokine Res. 9:607.

## CITATIONS:

1. Uzawa A, Mori M et al. Cytokine and chemokine profiles in neuromyelitis optica: significance of interleukin-6. Mult Scler. 2010 Dec;16(12):1443-52.
2. Klaus W Frommer et al. IGF-independent effects of IGFBP-2 on the human breast cancer cell line Hs578T. J Mol Endocrinol August 1, 2006 37 13-23.
3. LIN-LIN WANG, PING-HUA TANG et al. Expression of CD39 mRNA is altered in the peripheral blood of patients with allergic asthma. Biomed Rep. Jan 2014; 2(1): 75–78.
4. David E. Joyner et al. Potential for Modulation of the Fas Apoptotic Pathway by Epidermal Growth Factor in Sarcomas. Sarcoma. Volume 2011 (2011), Article ID 847409, 15 pages.