> TGF-β1 EIA kit

Catalog # ADI-900-155

96 Well Enzyme Immunoassay Kit
For use with plasma, serum and tissue culture supernatants

All reagents, except standard, should be stored at 4˚C. Store standard at -20˚C.

Check our website for additional protocols, technical notes and FAQs.

For proper performance, use the insert provided with each individual kit received.

Table of Contents

1. Sample Antigen

Well Coated with Protein G
Affinity Purified Antigen Specific Antibody

1) Incubate

2.

1) Wash
2) Add Antibody
3) Incubate

3.

1) Wash
2) Add HRP Conjugate
3) Incubate

4.

1) Wash
2) Add Substrate
3) Incubate

5.

COLOR

Measure at 450 nm.
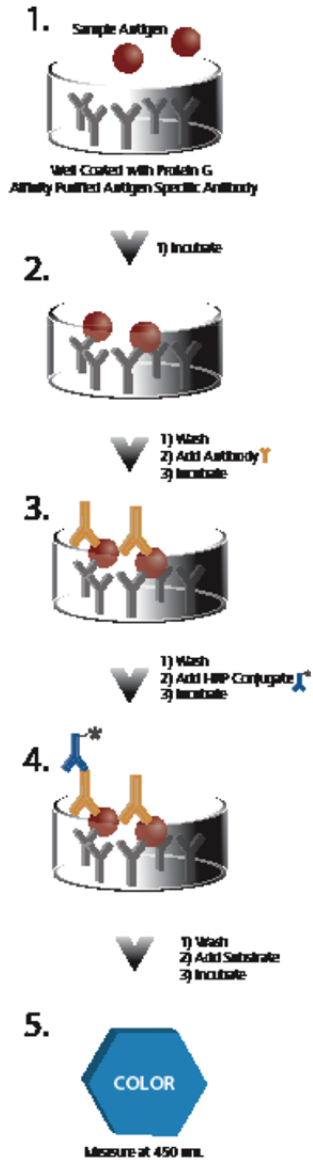
Introduction

Transforming growth factor beta 1 is also known as TGFβ1, TGF β, and TGFB. The inactive form consists of a TGF-β1 homodimer that is non-covalently linked to a latency-associated peptide homodimer. The active form is a homodimer of mature TGF-β1 that is disulfide linked. The precursor is cleaved into mature TGF-β1 and the latency-associated peptide[1, 2]. Many cells can synthesize TGF-β1, which is secreted to function in proliferation, differentiation, transformation, signaling and apoptosis. This cytokine has been implicated in many diseases including diabetes[3], renal disease[4], chronic pulmonary obstructive disease[5], as well as many cancers including prostate[6], and colon[7]. This protein has generated tremendous excitement as a potential tumor-specific cancer therapeutic. Specifically, as a stable soluble dimer, it selectively induces apoptosis in many transformed cells but not in normal cells[8]. The literature contains many recent reviews on the biology and function of TGF-β1[4, 6, 9, 10].

Principle

1.   Samples and standards are added to wells coated with a monoclonal antibody specific for TGF-β1. The plate is then incubated.

2.   The plate is washed, leaving only bound TGF-β1 on the plate. A yellow solution of polyclonal antibody to TGF-β1 is then added. This binds the TGF-β1 captured on the plate. The plate is then incubated.

3.   The plate is washed to remove excess antibody. A blue solution of HRP conjugate is added to each well, binding to the TGF-β1 polyclonal. The plate is again incubated.

4.   The plate is washed to remove excess HRP conjugate. TMB Substrate solution is added. The substrate generates a blue color when catalyzed by the HRP.

5.   Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is directly proportional to the level of TGF-β1 in the sample

Materials Supplied

1. Assay Buffer 13
    110 mL, Catalog No. 80-1627
    Tris buffered saline containing protein and detergents.

2. Assay Buffer 30
    110 mL, Catalog No. 80-1610
    Tris buffered saline and urea containing detergents.

3. human TGF-β1 Standard
    0.25 mL, Catalog No. 80-1577
    One vial containing 10,000 pg/mL of recombinant human TGF-β1.

4. TGF-β1 Clear Microtiter Plate
    One Plate of 96 Wells, Catalog No. 80-1574
    A plate of break-apart strips coated with a mouse monoclonal antibody specific to TGF-β1.

5. Wash Buffer Concentrate
    100 mL, Catalog No. 80-1287
    Tris buffered saline containing detergents.

6. human TGF-β1 EIA Antibody
    10 mL, Catalog No. 80-1575
    A yellow solution of polyclonal antibody to TGF-β1.

7. TGF-β1 EIA Conjugate
    10 mL, Catalog No. 80-1576
    A blue solution of streptavidin conjugated to horseradish peroxidase.

8. TMB Substrate
    10 mL, Catalog No. 80-0350
    A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide.

9. Stop Solution 2
    10 mL, Catalog No. 80-0377
    A 1N solution of hydrochloric acid in water.

10. TGF-β1 Assay Layout Sheet
    1 each, Catalog No. 30-0235

11. Plate Sealer
    3 each, Catalog No. 30-0012

Storage

All components of this kit, except the TGF-β1 Standard, are stable at 4°C until the kit's expiration date. The TGF-β1 Standard must be stored at -20°C .

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 50 μL and 1,000 μL.
3. Repeater pipet for dispensing 100 μL.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Lint-free paper for blotting.
8. Microplate reader capable of reading at 450 nm.
9. Graph paper for plotting the standard curve.
10. Glacial acetic acid (A.C.S. Grade, 17.4N).
11. HEPES, free acid (Reagent Grade, M.W. 238.8).
12. Urea (Reagent Grade, M.W. 60.06).
13. Sodium hydroxide (A.C.S. Grade, 10N).
14. Hydrochloric acid (A.C.S. Grade, I2N).

Reagent Preparation

1. **Wash Buffer**

   Prepare the wash buffer by diluting 50 mL of the supplied Wash Buffer Concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. **Activation Reagent**

   For cell culture supernatant

   1N HCl, 50 mL

   To 25 mL deionized water, slowly add 4.17 mL 12N HCl, mix, adjust volume to 50 mL using deionized water.

   1.2N NaOH, 0.5M HEPES, 50 mL

   To 30 mL deionized water, slowly add 6 mL 10N NaOH, mix, then add 5.95 g HEPES, stir well until all goes into solution. Bring final volume to 50 mL using deionized water.

   For Serum/Plasma

   **2.5N Acetic Acid, 10M Urea, 50 mL**

   In a glass beaker, add 20 mL deionized water, then add 30.04 g urea, mix until urea dissolves. Slowly add 7.18 mL glacial acetic acid, mix well, bring final volume to 50 mL using deionized water. * The buffer can be stored at room temperature. When urea comes out of solution, place the bottle containing buffer into a 37°C water bath until dissolved.

   2.7N NaOH, 1M HEPES, 50 mL

   Slowly add 13.5 mL 10N NaOH to 25 mL deionized water, mix well. Add 11.9 g HEPES, mix well until HEPES dissolves. Bring final volume to 50 mL with deionized water.

3. **human TGF-β1 Standards**

   Thaw standard on ice. Use the appropriate assay buffer based on the sample that will be tested. Use Assay Buffer 13 for activated cell culture supernatants and Assay Buffer 30 for activated serum/plasma samples.
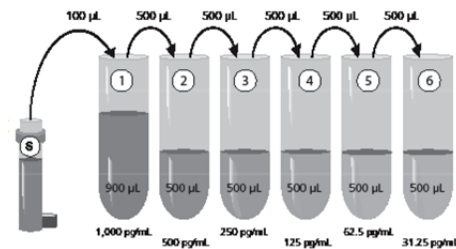
   Label six 12 x 75 mm polypropylene tubes #1 through #6. Pipet 900 µL of the appropriate assay buffer into tube #1. Pipet 500 µL of the assay buffer into tubes #2 through #6. Add 100 µL of the 10,000 pg/mL standard stock into tube #1 and vortex thoroughly. Add 500 µL of tube #1 to tube #2 and vortex thoroughly. Add 500 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #6.

   Diluted standards should be used within 60 minutes of preparation.
   The concentration of TGF-β1 in tubes is labeled above.

Sample Handling

TGF-β1, *in vivo*, is processed from a latent form to the bioactive form of the protein. Only the bioactive form is immunoreactive and detected in the kit. This process can be performed *in vitro*.

Serum supplements for cell culture contain TGF-β1. It's recommended that a sample of serum-supplemented cell culture media be analyzed as a control or cell culture can be performed with serum-free media if possible.

To measure circulating levels of TGF-β1 platelet-poor plasma should be used and care taken to avoid platelet degranulation. Platelets constitute a major source of TGF-β1.

Sample Activation

Cell Culture Supernates
1. Add 20 μL of 1N HCl to 100 μL culture supernate.
2. Mix well. Incubate 10 minutes at room temperature.
3. Neutralize the sample with 20 μL 1.2N NaOH/0.5M HEPES.
4. Mix well and assay immediately after dilution with assay buffer.

   Sample is now at a 1:1.4 dilution and requires an additional 1:2 dilution with **Assay Buffer 13** before runnning the assay (mix 140 μL activated sample with 140 μL Assay Buffer 13) . This will bring the samples to a final dilution of 1:2.8. A minimum of a 1:2.8 final dilution is required to eliminate matrix interference in the assay.

Serum or Plasma
1. Add 50 μL 2.5N acetic acid/10M urea to 50 μL serum / plasma.
2. Mix well. Incubate 10 minutes at room temperature.
3. Neutralize the sample with 50 μL 2.7N NaOH/1M HEPES.
4. Add 350 μL Assay Buffer 13.

   Sample is now at a 1:10 dilution and requires an additional 1:12 dilution with **Assay Buffer 30** before running in the assay. This will bring the sample to a final dilution of 1:120. A minimum of a 1:120 final dilution is required to eliminate matrix interference in the assay.

Sample Diluents

Dilute the standards and activated samples in Assay Buffer 13 if activated cell culture supernatants will be tested in the assay. Otherwise use Assay Buffer 30 for the standard and activated serum/plasma samples.

Minimum Recommended Dilution After Sample Activation

Cell Culture supernatant	≥ 1:2
Serum	≥ 1:12
Plasma	≥ 1:12

Sample Recoveries

TGF-β1 concentrations were measured in activated-neutralized human serum samples. TGF-β1 was spiked into the activated samples and diluted with the assay buffer, then assayed in the kit.

The following results were obtained:

Sample	Recommended Dilution	%Recovery
Activated human serum	≥1:12	99.0%

Assay Procedure

Bring all reagents, except for the standard, to room temperature for at least 30 minutes prior to opening.

All standards, controls, and samples should be run in duplicate.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

Pipet the reagents to the sides of the wells to avoid possible contamination.

Prior to the addition of antibody, conjugate, and substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

1. Pipet 100 μL of the appropriate assay buffer into the S0 (0 pg/mL standard) wells.
2. Pipet 100 μL of Standards #1 through #6 to the bottom of the appropriate wells.
3. Pipet 100 μL of the samples to the bottom of the appropriate wells.
4. Seal the plate. Incubate for 1 hour on a plate shaker (~500 rpm) at room temperature.
5. Empty the contents of the wells and wash by adding 400 μL of wash buffer to every well. Repeat 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
6. Pipet 100 μL of yellow antibody into each well except the blank.
7. Seal the plate. Incubate for 2 hours on a plate shaker (~500 rpm) at room temperature.
8. Wash as above (Step 5).
9. Add 100 μL of blue conjugate to each well except the blank.
10. Seal the plate. Incubate for 30 minutes on a plate shaker (~500 rpm) at room temperature.
11. Wash as above (Step 5).
12. Pipet 100 μL of substrate solution into each well.
13. Incubate for 30 minutes on a plate shaker (~500 rpm) at room temperature.
14. Pipet 100 μL of stop solution into each well.
15. After blanking the plate reader against the substrate blank, read optical density at 450 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

Calculation of Results

Several options are available for the calculation of the concentration of TGF-β1 in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4-parameter logistic curve fitting program. If data reduction software is not readily available, the concentrations can be calculated as follows:

1. Calculate the average Net OD for each standard and sample by subtracting the average blank OD from the average OD for each standard and sample.

    Average Net OD = Average OD - Average Blank OD

2. Using linear graph paper, plot the average Net OD for each standard versus TGF-β1 concentration in each standard. Approximate a straight line through the points. The concentration of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Calibration

Calibration to the NIBSC/WHO TGF-β1 International Reference Reagent 87/514 has been determined. To convert sample values obtained in the TGF-β1 kit to this NIBSC/WHO TGF-β1 Standard, use the equation below.

NIBSC/WHO 87514 value (pg/mL) = Obtained human TGF-β1 value (pg/mL) x 0.915

Typical Results in Assay Buffer 13

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Net OD	TGF-β1 (pg/mL)
S0	0.029	0
S1	1.950	1,000
S2	1.128	500
S3	0.604	250
S4	0.321	125
S5	0.186	62.5
S6	0.111	31.25
Unknown 1	1.172	526
Unknown 2	0.326	125

Total TGF- β1 Conc (pg/ml)
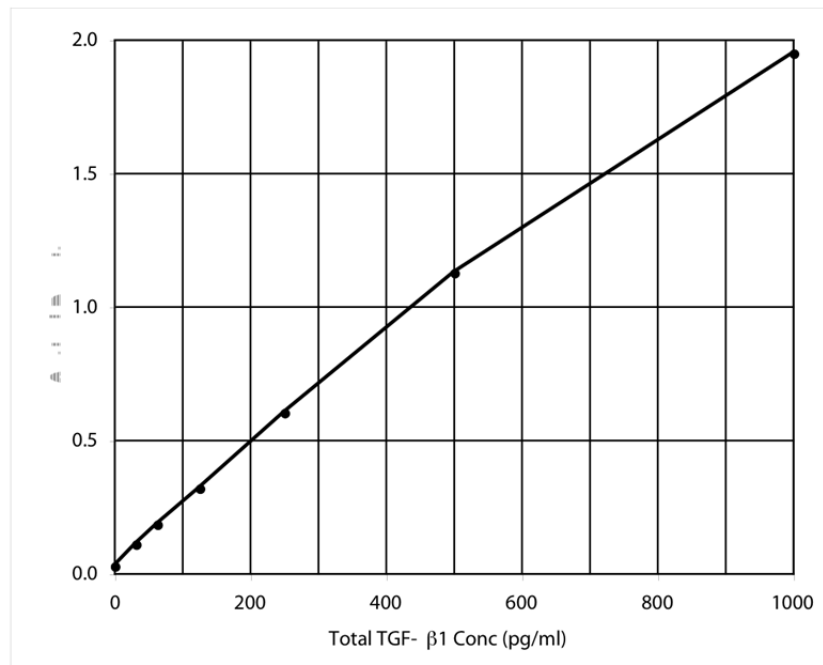
Typical Results in Assay Buffer 30

The results shown below are for illustration only and should not be used to calculate results from another assay.

Sample	Net OD	TGF-β1 (pg/mL)
S0	0.052	**0**
S1	1.780	**1,000**
S2	1.031	**500**
S3	0.515	**250**
S4	0.299	**125**
S5	0.175	**62.5**
S6	0.113	**31.25**
Unknown 1	1.003	**492**
Unknown 2	0.268	**115**
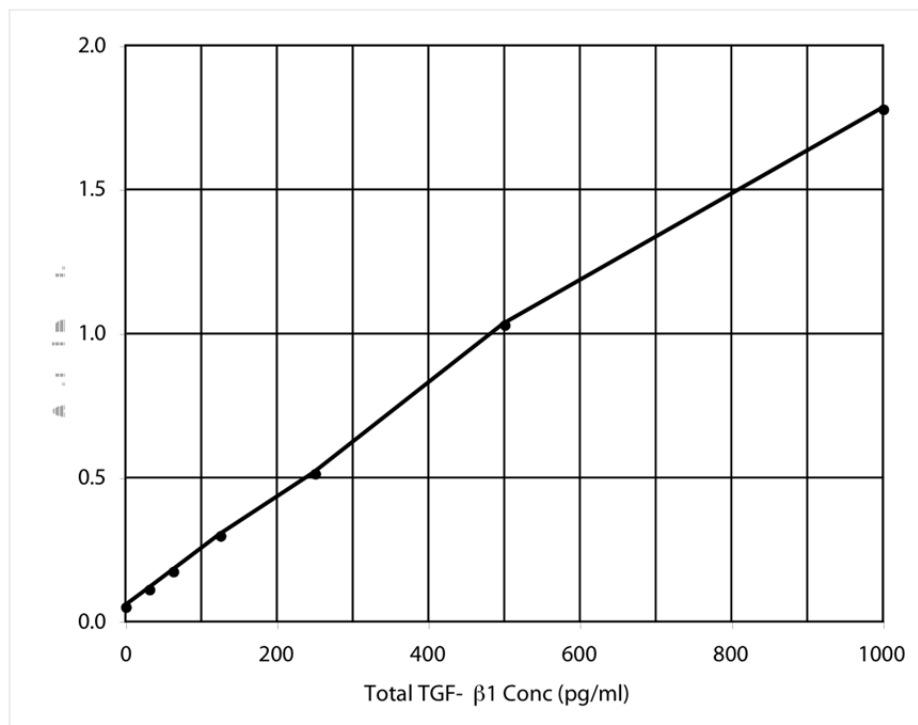
Performance Characteristics

Specificity

The cross reactivities for a number of related compounds were determined by diluting cross reactants in the assay buffer at three different concentrations of 10,000,000 pg/mL, 100,000 pg/mL and 10,000 pg/mL. These samples were then measured in the assay.

Compound	Cross Reactivity	
human TGF-β1	100%	(Assay Buffer 13 & Assay Buffer 30)
human TGF-β1.2	2.23%	(Assay Buffer 13)
	1.21%	(Assay Buffer 30)
human TGF-β2	<0.03%	(Assay Buffer 13 & Assay Buffer 30)
human TGF-β3	<0.03%	(Assay Buffer 13 & Assay Buffer 30)
human latent TGF-β1	<0.71%	(Assay Buffer 13)
	<0.38%	(Assay Buffer 30)
human LAP (latent associated protein)	<0.03%	(Assay Buffer 13 & Assay Buffer 30)

Sensitivity

The sensitivity of the assay, defined as the concentration of TGF-β1 measured at 2 standard deviations from the mean of 24 zeros along the standard curve, was determined to be 3.3 pg/mL in Assay Buffer 13, and 10.8 pg/mL in Assay Buffer 30.

Linearity

A buffer sample containing TGF-β1 was serially diluted 1:2 in Assay Buffer 13 and measured in the assay. The results are shown in the table below.

Dilution	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
Neat	------------	789 pg/mL	-----------
1:2	395 pg/mL	410 pg/mL	103.8 %
1:4	205 pg/mL	208 pg/mL	101.4 %
1:8	104 pg/mL	102 pg/mL	98.1 %
1:16	51 pg/mL	54 pg/mL	105.9 %
1:32	27 pg/mL	25 pg/mL	92.6 %

Another buffer sample containing TGF-β1 was serially diluted 1:2 in Assay Buffer 30 and measured in the assay. The results are shown in the table below.

Dilution	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
Neat	-------------	778 pg/mL	----------
1:2	389 pg/mL	368 pg/mL	94.6 %
1:4	184 pg/mL	185 pg/mL	100.5 %
1:8	93 pg/mL	105 pg/mL	112.9 %
1:16	52 pg/mL	52 pg/mL	100.0 %
1:32	26 pg/mL	28 pg/mL	107.7 %

Precision

Intra-assay precision was determined by assaying 24 replicates of three buffer controls containing TGF-β1 in a single assay.

In Assay Buffer 13   **In Assay Buffer 30**

pg/mL	%CV
98	8.5
251	3.4
475	3.9

Inter-assay precision was determined by measuring buffer controls of varying TGF-β1 concentrations in multiple assays over several days.

In Assay Buffer 13   **In Assay Buffer 30**

pg/mL	%CV
110	9.4
250	5.9
481	5.3

References

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Notes

Use of Product

This product contains research chemicals. As such, they should be used and handled only by or under the supervision of technically qualified individuals. This product is not intended for diagnostic or human use.

Warranty

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Global Headquarters

Enzo Life Sciences Inc.	**Enzo Life Sciences (ELS) AG**
10 Executive Blvd	Industriestrasse 17, Postfach
Farmingdale, NY 11735	CH-4415 Lause / Switzerland
(p) 1-800-942-0430	(p) +41/0 61 926 89 89
(f) 1-631-694-7501	(f) +41/0 61 926 89 79
(e) info-usa@enzolifesciences.com	(e) info-ch@enzolifesciences.com

Please visit our website at www.enzolifesciences.com for additional contact information.