ELISA Kit

Catalog # KAC1688 (96 tests)

Multispecies

TGF-β1

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INTRODUCTION

Transforming growth factor-alpha (TGF-α) and transforming growth factor-beta (TGF-β) have been implicated in diverse physiologic and pathophysiologic functions including immunological, inflammatory, and neoplastic processes. TGF-β is one of the most potent immunoregulatory molecules known. It is a 25,000-dalton homodimeric protein, with three known isoforms in man, TGF-β1, TGF-β2 and TGF-β3. TGF-β's are synthesized and secreted by various transformed and normal cells including lymphocytes and monocytes.

Differential expression of the isoforms of TGF-β is controlled both *in vivo* and *in vitro*. Selective regulation of expression of the TGF-β's is under the control of several factors, including oncogenes and tumor suppressor genes. In addition to transcriptional control, TGF-β's appear to be regulated posttranscriptionally. TGF-β1 has a special importance in immunoregulation. Further evidence for the differential expression of TGF-β isoforms comes from studies of osteosarcoma. In osteosarcoma, the expression of TGF-β3, but not TGF-β2 or TGF-β1, is highly correlated with disease progression (1-3).

PURPOSE

The Invitrogen Multispecies Transforming Growth Factor-beta 1 (Multispecies TGF-β1) ELISA is to be used for the quantitative determination of TGF-β1 in human, mouse, rat and swine serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant TGF-β1.

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Read entire protocol before use.

PRINCIPLE OF THE METHOD

The Invitrogen Multispecies TGF-β1 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for TGF-β1 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known TGF-β1 content, control specimens, and extracted unknowns, are pipetted into these wells, followed by the addition of a biotinylated second antibody.

During the first incubation, TGF-β1 antibody binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess detection antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of TGF-β1 present in the original specimen.

REAGENTS PROVIDED

Note: *Store all reagents at 2 to 8°C.*

Reagent	***96 Test Kit***
TGF-β1 Standard, recombinant human TGF-β1. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer. Contains 8 mM sodium azide; 25 mL per bottle.	1 bottle
Antibody Coated Wells; 96 wells per plate.	1 plate
Extraction Solution; 25 mL per bottle.	1 bottle
TGF-β1 Biotin Conjugate, (Biotin-labeled anti-TGF-β1). Contains 8 mM sodium azide; 5.5 mL per bottle.	1 bottle
Streptavidin-Peroxidase (HRP) (100x)concentrate. Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial
Streptavidin-Peroxidase (HRP) Diluent. Contains 0.05% Proclin 300; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25X). 100 mL per bottle.	1 bottle
Stabilized Chromogen, Tetramethylbenzidine (TMB). 25 mL per bottle.	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	3

Disposal Note: This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
6. Polypropylene test tubes for standard dilution and sample extraction.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.

SAMPLE COLLECTION AND STORAGE

A. Cell culture media

Centrifuge at 1000 x g for 10 minutes, before extraction step to eliminate any particulates.

B. Serum

Collect blood by venipuncture, taking care to avoid hemolysis.

Separate the serum from the cells within 1 hour. Centrifuge at 1000 x g for 10 minutes at 4°C. Collect the serum.

C. Plasma

It is very important to prevent platelet degranulation during the plasma collection because platelets constitute one of the main sources of TGF-β1.

Heparin may be used as anticoagulant.

Separate the plasma from the cells within 1 hour. Centrifuge at 1000 x g for 10 minutes, at 4°C. Collect the plasma.

Caution: the following step is for obtaining platelet depleted plasma and should be performed immediately prior to the extraction step.

Centrifuge the plasma at 3000 x g for 10 minutes (4°C) to eliminate platelets. Collect platelet-depleted plasma.

D. Storage of samples

If samples (cell culture medium, serum, heparin plasma) are not assayed immediately, but within one month following collection, store frozen at –20°C. For longer storage, freeze at –70°C. Avoid repetitive freeze-thaw cycles.

SAMPLE EXTRACTION *This step allows for the release of TGF-β1 from latent complexes, making it accessible for measurement in the immunoassay.*

A. Cell culture media

In a polypropylene tube, add:

0.25 mL of each cell culture medium sample.

0.05 mL *Extraction Solution*.

Vortex. Incubate 30 minutes at 4°C. Add 0.25 mL *Standard Diluent Buffer*. After this extraction step, cell culture media are diluted 2.2-fold. Note: since serum supplements for cell culture may contain TGF-β1, it is recommended that a sample of appropriate serum supplemented cell culture medium be analyzed as a control.

A limited study was undertaken to determine the impact of serum supplements on measured TGF-β1 concentration. Non-inactivated FCS (ICN, cat: 092910154) contained less than 5000 pg/mL TGF-β1, while inactivated FCS contained between 20 and 150 pg/mL TGF-β1. These data are for illustration only and should never be used in the place of actual data.

B. Serum and plasma

In a polypropylene tube, add:

0.1 mL of each serum or plasma sample.

0.3 mL of *Extraction Solution*.

Vortex. Incubate 30 minutes at room temperature with vigorous continuous shaking. Centrifuge at 1000 x g for 10 minutes. Dilute the supernatant 10-fold with *Standard Diluent Buffer*, in polypropylene tubes. (Example: 50 µL with 450 µL.)

After this extraction step, samples are diluted 40-fold.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected and extracted as described previously.
4. It is recommended that all standards, controls and samples be run in duplicate.
5. Samples that are >2000 pg/mL should be diluted with *Standard Diluent Buffer*.
6. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
7. Cover or cap all reagents when not in use.
8. **Do not mix or interchange different reagent lots from various kit lots**.
9. Do not use reagents after the kit expiration date.
10. Read absorbances within 2 hours of assay completion.
11. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.

12. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
13. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed.

REAGENT PREPARATION AND STORAGE

A. Reconstitution and Dilution of TGF–β1 Standard

This assay has been calibrated against the WHO reference preparation 89/514 (NIBSC, Hertfordshire, UK, EN6 3QG). One microgram equals 14,600 arbitrary units.

Note: **Polypropylene tubes** may be used for standard dilutions.

1. Reconstitute the TGF-ß1 Standard with *Standard Diluent Buffer* sufficient to obtain a concentration of 10,000 pg/mL. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use standard within 1 hour of reconstitution. Add 0.2 mL of the 10,000 pg/mL into 0.8 mL of *Standard Diluent Buffer* to create a 2000 pg/mL Standard.
2. Add 0.500 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 1000, 500, 250, 125, 62.5 and 31.2 pg/mL TGF-β1.
3. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of TGF-β1 Standard

Standard:	Add:	Into:
2000 pg/mL	Prepare as described in Step 1.	
1000 pg/mL	0.500 mL of the 2000 pg/mL std.	0.500 mL of the Diluent Buffer
500 pg/mL	0.500 mL of the 1000 pg/mL std.	0.500 mL of the Diluent Buffer
250 pg/mL	0.500 mL of the 500 pg/mL std.	0.500 mL of the Diluent Buffer
125 pg/mL	0.500 mL of the 250 pg/mL std.	0.500 mL of the Diluent Buffer
62.5 pg/mL	0.500 mL of the 125 pg/mL std.	0.500 mL of the Diluent Buffer
31.2 pg/mL	0.500 mL of the 62.5 pg/mL std.	0.500 mL of the Diluent Buffer
0 pg/mL	0.500 mL of the Diluent Buffer	An empty tube

Discard all remaining reconstituted and diluted standards after completing assay. Return the *Standard Diluent Buffer* to the refrigerator.

C. Storage and Final Dilution of Streptavidin-HRP

1. Dilute 10 µL of this 100x concentrated solution with 1 mL of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Streptavidin-HRP Concentrate	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused *Streptavidin-HRP* concentrate to the refrigerator.

D. Dilution of Wash Buffer

Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 200 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 200 µL of standards, samples (after extraction) or controls to the appropriate microtiter wells. (See **REAGENT PREPARATION AND STORAGE**, Section B.)
4. Pipette 50 µL of biotinylated anti-TGF-β1 (*Biotin Conjugate*) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix thoroughly.

5. Cover plate with *plate cover* and incubate for **3 hours at room temperature**.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
7. Add 100 μL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE,** Section C.)
8. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
10. Add 100 μL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
11. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.

12. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
13. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
14. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
15. Read the TGF-β1 concentrations for unknown extracted samples and controls from the standard curve plotted in step 14. **Multiply results by a factor of 40 (for sera and plasma samples) or by a factor of 2.2 (for cell culture media samples).** Samples producing signals greater than that of the highest standard (2000 pg/mL) should be diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 2000 pg/mL TGF-β1.

Standard TGF-β1 (pg/mL)	Optical Density (450 nm)
0	0.167
	0.165
31.2	0.241
	0.239
62.5	0.312
	0.308
125	0.522
	0.465
250	0.688
	0.657
500	1.087
	1.074
1000	1.767
	1.773
2000	2.895
	2.954

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 2000 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >2000 pg/mL with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum or plasma samples have not been thoroughly investigated. The rate of degradation of native TGF-β1 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of TGF-β1 is <15.6 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 24 times.

PRECISION

1. Intra-Assay Precision

Samples of known human TGF-β1 concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	183.9	577.66	1537
SD	10.19	32.94	96.7
%CV	5.5	5.7	6.2

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

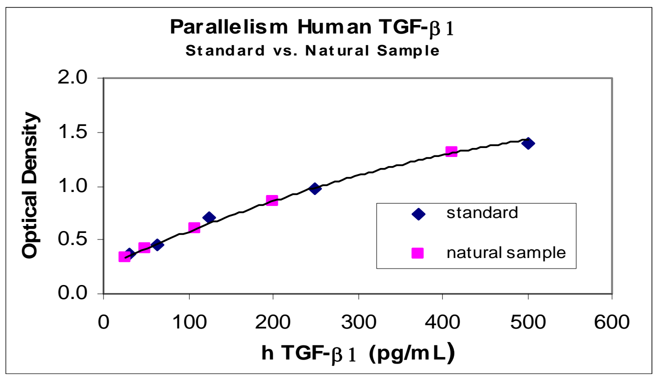
Samples were assayed 40 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	181.02	564.1	1547.9
SD	13.67	37.1	123.3
%CV	7.5	6.6	7.9

SD = Standard Deviation
CV = Coefficient of Variation

PARALLELISM

Natural human TGF-β1 was serially diluted in *Standard Diluent Buffer*. The optical density of each dilution was plotted against the standard curve. Parallelism between the natural and recombinant protein is demonstrated by the figure below and indicates that the standard accurately reflects natural human TGF-β1 content in samples.



LINEARITY OF DILUTION

An extracted human serum containing 700 pg/mL of measured TGF-β1 was serially diluted in *Standard Diluent Buffer* over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

RECOVERY

The recovery of TGF-β1 added to normal human serum, human heparinized plasma and human EDTA plasma averaged 96.8%, 89.2% and 99.1%, respectively. The recovery of TGF-β1 added to tissue culture medium containing 10% fetal calf serum averaged 93.3%, while the recovery of TGF-β1 added to tissue culture medium containing 1% fetal calf serum averaged 99.1%.

SPECIFICITY

The Invitrogen Multispecies TGF-β1 kit can be used to measure natural human, porcine, mouse, and rat TGF-β1.

Buffered solutions of a panel of substances at 50,000 pg/mL were assayed with the Invitrogen Multispecies TGF-β1 Kit. The following substances were tested and found to have no cross-reactivity: human IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, GM-CSF, TNF-α, TNF-β, IFN-α, IFN-β, IFN-γ, TGF-β2, OSM, MIP-1α, MIP-1β, LIF, MCP-1, G-CSF and RANTES.

EXPECTED VALUES

Each laboratory must establish its own normal values. For guidance, 16 normal sera gave values between 19 ng/mL and 71 ng/mL.

REFERENCES

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2. Kulkarni, A.B. and S. Karlsson (1993) Am. J. Pathol. 143(1):3-9.
3. Kloen, P., et al. (1997) Cancer 80(12):2230-2239.

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Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
Protect from Light	Protect from light		Consult accompanying documents
i	Directs the user to consult instructions for use (IFU), accompanying the product.		

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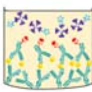
Multispecies TGF-β1 Assay Summary

Add 200 μL of standards or controls & samples
(after extraction)

Add 50 μL of Biotin Conjugate
Incubate for 3 hours at RT

aspirate and wash 4x

Incubate 100 μL of Streptavidin-HRP
Working Solution for 30 minutes at RT



aspirate and wash 4x

Incubate 100 μL of Stabilized Chromogen
for 30 minutes at RT

Add 100 μL of Stop Solution
and read at 450 nm

Total time: 4 hours