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R&D SYSTEMS

Quantikine[™] ELISA

Human/Mouse/Rat/Porcine/Canine TGF- β 1 Immunoassay

Catalog Number DB100C
SB100C
PDB100C

For the quantitative determination of activated human/mouse/rat/porcine/canine Transforming Growth Factor beta 1 (TGF- β 1) concentrations in cell culture supernates, serum, platelet-poor plasma, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Human TGF- β 1 is a 25 kDa, disulfide-linked homodimeric protein involved in a number of key developmental, immunologic, and homeostatic processes (1-4). The molecule is synthesized as a 390 amino acid (aa) precursor that contains a 23 aa signal sequence, a 255 aa pro-region, and a 112 aa mature segment. Processing of the molecule is complex, and it is generally secreted as a latent form (5). Prior to release, the prepro-form is cleaved of its signal sequence, followed by glycosylation of its pro-region. The glycosylation process includes the unusual attachment of mannose-6 phosphate residues. This is followed by furin convertase-mediated cleavage of the prohormone, creating an 80 kDa disulfide-linked proregion (termed LAP for latency-associated protein), plus a 25 kDa disulfide-linked mature segment (termed TGF- β 1) (6-8). These two independent disulfide-linked polypeptides associate in a non-covalent interaction that renders TGF- β 1 inactive. Although direct secretion of this 80K:25K complex can occur, it does so inefficiently. To facilitate secretion plus extracellular storage, a third 200 kDa component termed LTBP is covalently-linked to the N-terminus of one of the two LAP polypeptide chains. This promotes secretion and subsequent storage within the extracellular matrix (9, 10). After secretion, TGF- β 1, via LTBP, covalently links to ECM. This complex is later cleaved by proteases and released, exposing mannose residues on LAP. It is postulated that exposed LAP mannose residues now are able to bind to cell surface IGF-II R, where dissociative events disrupt the LAP-TGF- β 1 complex. This results in the release of active, homodimeric TGF- β 1 (7, 10). Mature mouse TGF- β 1 shares 100% aa sequence identity with rat and cotton rat TGF- β 1 (11, 12), 99% aa identity with human, canine, and porcine TGF- β 1 (13, 14, 15), and 97% aa identity with guinea pig TGF- β 1 (16). Relative to mouse TGF- β 2 and β 3, mature mouse TGF- β 1 shares 72% and 78% aa sequence identity, respectively (17, 18).

The traditional high-affinity receptor for TGF- β 1 is a heteromeric complex consisting of transmembrane serine/threonine kinases. Two types are involved; a constitutively phosphorylated, ligand-binding 80 kDa glycoprotein termed TGF- β 1 RII and a signal-transducing, non-ligand-binding 55 kDa glycoprotein termed TGF- β RI/ALK-5 (19-22). It is suggested that TGF- β 1 first binds TGF- β 1 RII, which then initiates a cross-phosphorylation of TGF- β 1 RI, culminating in signal transduction. There is also a third TGF- β receptor termed TGF- β 1 RIII, which can be either the 250 kDa proteoglycan named betaglycan, or the 180 kDa glycoprotein termed endoglin/CD105 (23, 24). It has been proposed that TGF- β 1 RIII captures TGF- β and "passes" it to TGF- β 1 RII (20). This is perhaps true for betaglycan but not endoglin. Endoglin does not bind TGF- β by itself; only within the context of TGF- β 1 RII ligand binding. Evidence suggests that rather than "passing" on ligand, endoglin may actually enter the receptor complex and modulate TGF- β downstream signaling (25, 26). Finally, and although ALK-5 has traditionally been assumed to be the only type I signaling receptor for TGF- β 1, it is also possible that ALK-1 may serve as a condition-dependent, type I TGF- β receptor (27).

TGF- β 1 has a wide range of activities. During an immune response, TGF- β 1 impacts antibody production by preferentially inducing IgA production in both mouse and human (28). It also regulates dendritic cell chemotaxis by altering the expression of chemokine receptors (29). Finally, it can downmodulate an inflammatory response by dampening macrophage activity and proinflammatory cytokine secretion (30). During wound healing, TGF- β 1 is released from activated platelets. This local source of TGF- β 1 has marked stimulatory effects on fibroblasts, where it induces matrix synthesis; on monocytes, where it induces proinflammatory mediator and growth factor secretion; and on keratinocytes, where it may promote keratinocyte proliferation by downmodulating its own signaling pathway (31). Finally, during development, TGF- β 1 may play a role in endochondral ossification, and its absence results in severely defective yolk sac vasculogenesis and hematopoiesis (32, 33).

The Quantikine™ Human/Mouse/Rat/Porcine/Canine TGF- β 1 Immunoassay is a 4.5 hour solid phase ELISA designed to measure TGF- β 1 in cell culture supernates, serum, platelet-poor plasma, and urine. It contains CHO cell-expressed recombinant TGF- β 1 and has been shown to quantitate the recombinant factor accurately. Results obtained using natural TGF- β 1 showed linear curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that this kit can be used to determine relative mass values for natural TGF- β 1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TGF- β 1 has been pre-coated onto a microplate. Standard and samples are pipetted into the wells and any TGF- β 1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TGF- β 1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of TGF- β 1 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.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the assay diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, further dilute the activated samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine™ Immunoassay, the possibility of interference cannot be excluded.

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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DB100C	CATALOG # SB100C	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
TGF-β1 Microplate	891124	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for TGF-β1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
TGF-β1 Standard	891126	2 vials	12 vials	Recombinant TGF-β1 in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Use a new standard for each assay. Discard after use.
TGF-β1 Conjugate	893003	1 vial	6 vials	12 mL/vial of a polyclonal antibody specific for TGF-β1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	1 vial	3 vials	12 mL/vial of a buffered protein base with preservatives. <i>For cell culture supernate/urine samples.</i>	
Assay Diluent RD1-73	895541	1 vial	3 vials	12 mL/vial of a buffered base with preservatives. <i>For serum/platelet-poor plasma samples.</i>	
Calibrator Diluent RD6-11	895489	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. <i>Use diluted 1:2 in this assay.</i>	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
Color Reagent A	895000	1 vial	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

DB100C contains sufficient materials to run ELISAs on one 96 well plate.

SB100C (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDB100C). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
TGF- β 1 Microplate	891124	50 plates
TGF- β 1 Conjugate	893003	50 vials
TGF- β 1 Standard*	891126	50 vials
Assay Diluent RD1-21	895215	25 vials
or		
Assay Diluent RD1-73	895541	25 vials
Calibrator Diluent RD6-11	895489	50 vials
Color Reagent A	895000	25 vials
Color Reagent B	895001	25 vials
Wash Buffer Concentrate	895126	9 bottles
Stop Solution	895174	25 vials
Plate Sealers	N/A	100 sheets
Package Insert	753376	2 booklets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

ADDITIONAL REAGENTS REQUIRED

For sample activation: *The reagents listed below are available as Sample Activation Kit 1 (R&D Systems®, Catalog # DY010).*

- Hydrochloric acid (A.C.S. Grade, 12 N)
- Sodium hydroxide (A.C.S. Grade, 10 N)
- HEPES, free acid (Reagent Grade, M.W. 238.3)

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 100 mL and 500 mL graduated cylinders
- **Polypropylene** test tubes for dilution of standards and activation of samples
- TGF- β 1 Controls (optional; R&D Systems, Catalog # QC01-1)

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Activated samples must be assayed immediately. Do not freeze activated samples.

Cell Culture Supernates - Remove particulates by centrifugation and assay (see activation procedure) immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Animal serum used in the preparation of cell culture media may contain high levels of latent TGF- β 1. For best results, do not use animal serum for growth of cell cultures when assaying for TGF- β 1 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- β 1.*

Human Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature. Centrifuge for 15 minutes at 1000 x g. Remove serum and assay (see activation procedure) immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Non-human Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay (see activation procedure) immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE COLLECTION & STORAGE *CONTINUED*

Human Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately (see activation procedure) or aliquot and store at ≤ -20 °C. Avoid repeated freeze/thaw cycles.

Note: *Neat unactivated urine samples exhibit a decrease in TGF- β 1 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.*

Platelet-poor Plasma* - Collect plasma on ice using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes 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 ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

***TGF- β 1 is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of TGF- β 1, platelet-poor plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical and Laboratory Standards Institute (CLSI), result in incomplete removal of platelets from plasma. 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- β 1 levels.**

ACTIVATION REAGENT PREPARATION

To activate latent TGF- β 1 to the immunoreactive 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.

Caution: *Wear protective clothing and safety glasses during preparation and use of these reagents. Refer to the appropriate SDS before use.*

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/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 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.

TGF-β1 SAMPLE ACTIVATION PROCEDURE

To activate latent TGF-β1 to immunoreactive TGF-β1 detectable by the Quantikine™ TGF-β1 immunoassay, 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 or optional controls (R&D Systems®, Catalog # QC01-1). The standards and controls contain active recombinant TGF-β1.

Cell Culture Supernates/Urine	Serum/Platelet-poor Plasma
To 100 µL of cell culture supernate/urine, add 20 µL of 1 N HCl.	To 40 µL of serum/platelet-poor plasma, add 10 µL of 1 N HCl.
Mix well.	Mix well.
Incubate 10 minutes at room temperature.	Incubate 10 minutes at room temperature.
Neutralize the acidified sample by adding 20 µL of 1.2 N NaOH/0.5 M HEPES.	Neutralize the acidified sample by adding 10 µL of 1.2 N NaOH/0.5 M HEPES.
Mix well.	Mix well.
Prior to the assay, dilute the activated sample with calibrator diluent. <i>See below for suggested dilutions.</i>	Prior to the assay, dilute the activated sample with calibrator diluent. <i>See below for suggested dilutions.</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 appropriate dilution factor.

Cell culture supernate samples (human and non-human) tested neat, but may require dilution if high endogenous levels are present (final dilution factor of sample is 1.4 if tested neat).

Human serum samples require a 40-fold dilution in Calibrator Diluent RD6-11 (diluted 1:2)[†]. A suggested 40-fold dilution is 10 µL of activated sample + 390 µL of Calibrator Diluent RD6-11 (diluted 1:2) (final dilution factor of sample is 60).

Human urine samples tested neat (final dilution factor of sample is 1.4).

Human platelet-poor plasma* samples require a 8-fold dilution in Calibrator Diluent RD6-11 (diluted 1:2)[†]. A suggested 8-fold dilution is 25 µL of activated sample + 175 µL of Calibrator Diluent RD6-11 (diluted 1:2) (final dilution factor of sample is 12).

Mouse and rat serum/platelet-poor plasma* samples require a 60-fold dilution in Calibrator Diluent RD6-11 (diluted 1:2)[†]. A suggested 60-fold dilution is 10 µL of activated sample + 590 µL of Calibrator Diluent RD6-11 (diluted 1:2) (final dilution factor of sample is 90).

Porcine serum samples require a 15-fold dilution in Calibrator Diluent RD6-11 (diluted 1:2). A suggested 15-fold dilution is 10 µL of activated sample + 140 µL of Calibrator Diluent RD6-11 (diluted 1:2) (final dilution factor of sample is 22.5).

Porcine platelet-poor plasma* samples require a 5-fold dilution in Calibrator Diluent RD6-11 (diluted 1:2). A suggested 5-fold dilution is 40 µL of activated sample + 160 µL of Calibrator Diluent RD6-11 (diluted 1:2) (final dilution factor of sample is 7.5).

Canine serum and platelet-poor plasma* samples require a 40-fold dilution in Calibrator Diluent RD6-11 (diluted 1:2). A suggested 40-fold dilution is 10 µL of activated sample + 390 µL of Calibrator Diluent RD6-11 (diluted 1:2) (final dilution factor of sample is 60).

*May require different dilutions depending on the extent of platelet contamination.

[†]See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

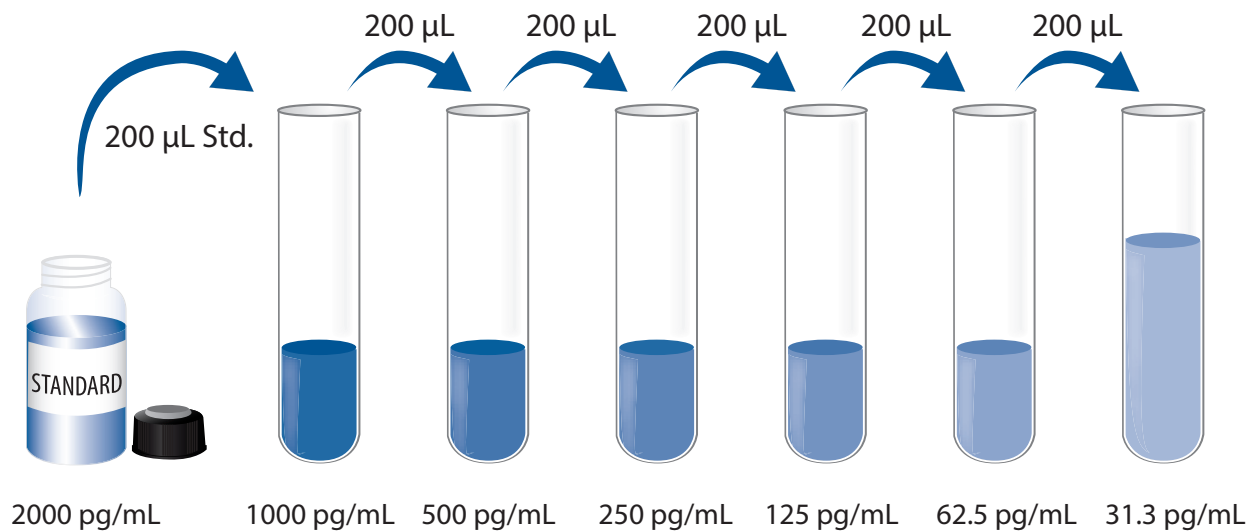
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD6-11 (diluted 1:2) - Add 20 mL of Calibrator Diluent RD6-11 to 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD6-11 (diluted 1:2).

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 μ L of the resultant mixture is required per well.

TGF- β 1 Standard - Refer to the vial label for reconstitution volume. Reconstitute the TGF- β 1 Standard with Calibrator Diluent RD6-11 (diluted 1:2). Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 μ L of Calibrator Diluent RD6-11 (diluted 1:2) into each tube. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted TGF- β 1 Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD6-11 (diluted 1:2) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, and activated samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 μ L of Assay Diluent RD1-21 (*for cell culture supernate/urine samples*) or Assay Diluent RD1-73 (*for serum/platelet-poor plasma samples*) to each well.
4. Add 50 μ L of standard, control, or activated sample* per well. Tap the plate gently for one minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 μ L) using a squirt bottle, manifold dispenser, or autowasher. 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.
6. Add 100 μ L of TGF- β 1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature **on the benchtop**.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μ L of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light**.
9. Add 100 μ L of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Activation Procedure.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

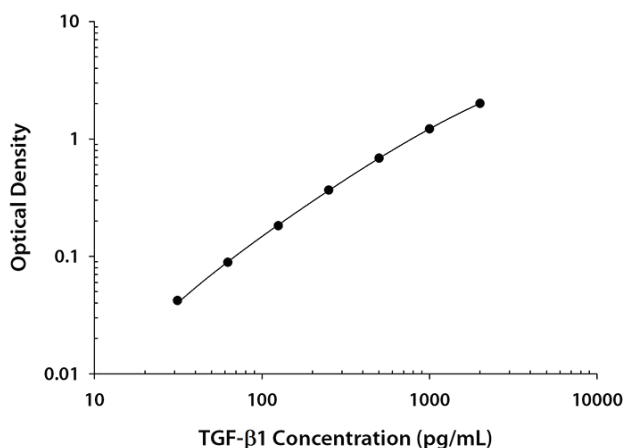
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the TGF- β 1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Since samples have been diluted in the activation step prior to the assay, the measured concentrations must be multiplied by the final dilution factor.

TYPICAL DATA

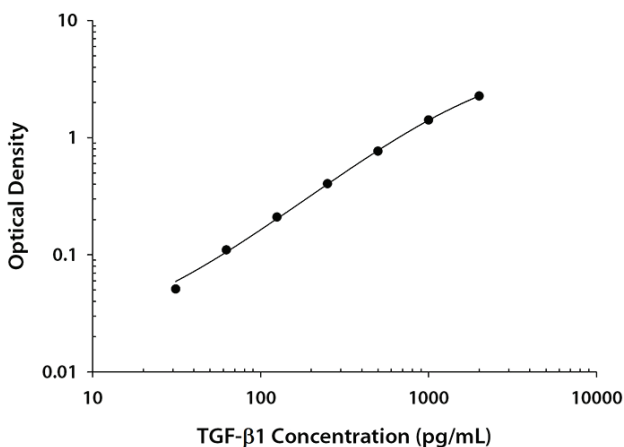
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.031	0.029	—
31.3	0.070 0.071	0.071	0.042
62.5	0.117 0.119	0.118	0.089
125	0.208 0.214	0.211	0.182
250	0.385 0.407	0.396	0.367
500	0.710 0.720	0.715	0.686
1000	1.243 1.252	1.248	1.219
2000	2.035 2.040	2.038	2.009

SERUM/PLATELET-POOR PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.013 0.014	0.014	—
31.3	0.064 0.065	0.065	0.051
62.5	0.123 0.124	0.124	0.110
125	0.218 0.230	0.224	0.210
250	0.413 0.423	0.418	0.404
500	0.774 0.785	0.780	0.766
1000	1.395 1.460	1.428	1.414
2000	2.249 2.307	2.278	2.264

PRECISION

Intra-Assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)

Three samples of known concentration were tested in separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE/URINE ASSAY

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	317	683	1271	312	657	1184
Standard deviation	11.3	42.2	31.1	26.1	46.6	92.3
CV (%)	3.6	6.2	2.4	8.4	7.1	7.8

SERUM/PLATELET-POOR PLASMA ASSAY

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	309	708	1072	303	622	1092
Standard deviation	15.3	40.0	44.3	21.2	47.2	70.0
CV (%)	5.0	5.6	4.1	7.0	7.6	6.4

RECOVERY

The recovery of TGF- β 1 spiked to levels throughout the range of the assay in activated samples was evaluated.

Sample Type	Average % Recovery	Range
Human urine (n=4)	114	105-123%
Human platelet-poor EDTA plasma (n=4)	90	77-107%
Human platelet-poor heparin plasma (n=4)	90	83-98%
Media + FBS (n=4)	103	80-125%
Serum-free media (n=3)	107	96-117%
Porcine platelet-poor EDTA plasma (n=4)	87	82-95%
Porcine platelet-poor heparin plasma (n=4)	85	76-95%

LINEARITY

To assess linearity of the assay, the activated samples containing and/or spiked with high concentrations of TGF- β 1, were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

Human Samples		Cell culture supernates (n=4)	Serum* (n=4)	Urine (n=4)	Platelet-poor	
					EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	100	97	93	105	105
	Range (%)	90-114	95-102	92-94	103-107	102-111
1:4	Average % of Expected	93	97	88	110	107
	Range (%)	82-117	94-104	83-91	108-113	103-112
1:8	Average % of Expected	93	95	88	112	109
	Range (%)	76-121	93-100	83-92	111-112	102-116
1:16	Average % of Expected	100	97	92	120	118
	Range (%)	85-124	92-104	86-101	115-124	110-125

*Samples were diluted after activation. See the Sample Activation Procedure.

Mouse Samples		Cell culture supernates (n=4)	Serum* (n=4)	Platelet-poor	
				EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	97	100	104	105
	Range (%)	91-102	92-105	90-115	103-111
1:4	Average % of Expected	90	100	106	107
	Range (%)	86-96	92-104	95-115	102-113
1:8	Average % of Expected	86	97	101	111
	Range (%)	79-95	91-101	88-110	100-119
1:16	Average % of Expected	85	96	99	109
	Range (%)	79-94	87-101	88-110	93-117

*Samples were diluted after activation. See the Sample Activation Procedure.

LINEARITY CONTINUED

Rat Samples		Cell culture supernates (n=2)	Serum* (n=4)	Platelet-poor	
				EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	107	100	102	104
	Range (%)	107-108	93-104	99-109	101-108
1:4	Average % of Expected	101	102	101	106
	Range (%)	100-103	99-107	96-104	104-110
1:8	Average % of Expected	97	100	103	107
	Range (%)	96-97	96-103	99-109	103-111
1:16	Average % of Expected	97	101	104	106
	Range (%)	96-98	96-108	97-115	97-113

*Samples were diluted after activation. See the Sample Activation Procedure.

Porcine Samples		Cell culture supernates (n=4)	Serum* (n=4)	Platelet-poor	
				EDTA plasma* (n=5)	Heparin plasma* (n=4)
1:2	Average % of Expected	97	103	106	110
	Range (%)	88-106	100-106	102-111	108-111
1:4	Average % of Expected	92	100	110	112
	Range (%)	81-102	97-106	109-111	108-115
1:8	Average % of Expected	94	99	112	117
	Range (%)	81-103	95-104	110-114	108-124
1:16	Average % of Expected	97	104	117	119
	Range (%)	86-106	97-109	115-118	109-124

*Samples were diluted after activation. See the Sample Activation Procedure.

Canine Samples		Cell culture supernates (n=3)	Serum* (n=4)	Platelet-poor	
				EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	99	100	98	100
	Range (%)	91-104	96-102	91-103	96-105
1:4	Average % of Expected	99	99	94	96
	Range (%)	96-102	95-103	87-98	87-107
1:8	Average % of Expected	100	100	92	98
	Range (%)	93-109	97-103	87-95	88-106
1:16	Average % of Expected	104	100	95	101
	Range (%)	95-120	95-107	86-105	96-106

*Samples were diluted after activation. See the Sample Activation Procedure.

SENSITIVITY

Thirty-three assays were evaluated and the minimum detectable dose (MDD) of human/mouse/rat/porcine/canine TGF- β 1 ranged from 0.889-5.50 pg/mL. The mean MDD was 2.38 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against highly purified CHO cell-expressed recombinant TGF- β 1 produced at R&D Systems®.

The NIBSC/WHO TGF- β 1 International Standard 89/514 (Human, rDNA Derived) was evaluated in this kit. The dose response curve of the reference reagent 89/514 parallels the Quantikine™ standard curve. To convert sample values obtained with the Quantikine Human/Mouse/Rat/Porcine/Canine TGF- β 1 kit to approximate NIBSC/WHO 89/514 Units, use the equation below:

Cell culture supernate/Urine - NIBSC/WHO 89/514 approximate value (IU/mL) = 0.0159 x Quantikine Human/Mouse/Rat/Porcine/Canine TGF- β 1 value (pg/mL)

Serum/Platelet-poor Plasma - NIBSC/WHO 89/514 approximate value (IU/mL) = 0.0171 x Quantikine Human/Mouse/Rat/Porcine/Canine TGF- β 1 value (pg/mL)

Note: *Based on data generated in May 2021.*

SAMPLE VALUES

Serum/Plasma/Urine - Samples were evaluated for the presence of TGF- β 1 in this assay.

Human Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=31)	51,640	32,091-95,147	11,901
Platelet-poor EDTA plasma (n=31)	2377	1414-4641	729
Platelet-poor heparin plasma (n=31)	2383	1445-3653	561
Urine* (n=10)	65.9	ND-108	—

*Only 40% of the urine samples measured detectable levels (> 31.3 pg/mL).

ND=Non-detectable

Mouse Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	97,352	78,751-114,130	12,623
Platelet-poor EDTA plasma (n=10)	41,763	16,577-68,505	16,887
Platelet-poor heparin plasma (n=10)	41,101	18,195-66,700	17,419

Rat Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	68,108	43,721-89,138	14,343
Platelet-poor EDTA plasma (n=5)	5833	4912-7820	1141
Platelet-poor heparin plasma (n=5)	13,971	5274-26,114	8392

Porcine Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=5)	17,597	13,481-23,914	4146
Platelet-poor EDTA plasma (n=5)	1955	1530-2810	518
Platelet-poor heparin plasma (n=5)	1567	1111-2475	551

Canine Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	33,455	2193-79,590	19,795
Platelet-poor EDTA plasma** (n=5)	2195	ND-2515	—
Platelet-poor heparin plasma*** (n=5)	3763	ND-4278	—

**Only 60% of the EDTA plasma samples measured detectable levels with the required 40 fold dilution. Two of five samples read just below the standard curve (< 31.3 pg/mL).

***Only 80% of the heparin plasma samples measured detectable levels with the required 40 fold dilution. One of five samples read just below the standard curve (< 31.3 pg/mL).

SAMPLE VALUES *CONTINUED*

Cell Culture Supernates:

Human peripheral blood mononuclear cells (PBMCs) were separated from whole blood by a density gradient centrifugation method using Ficoll-Paque Plus. CD4⁺ T cells were isolated from PBMCs using the MagCelect™ Human CD4⁺ T cell Isolation Kit (R&D Systems®, Catalog # MAGH102). Cells were seeded at 5×10^5 /mL and cultured using Excellerate™ Human T Cell Expansion Media, Xeno-Free (R&D Systems, Catalog # CCM030). T cells were left untreated or treated with 10 ng/mL GMP recombinant human (rh) IL-7 (R&D Systems, Catalog # 207-GMP), 10 ng/mL GMP rhIL-15 (R&D Systems, Catalog # 247-GMP), and stimulated via their T cell receptor (TCR) and co-stimulatory receptor for 5 days. TCR stimulation was mediated using 25 μ L Cloudz™ CD3/28 particles (Cloudz T Cell Activation Kit - CD3/CD28, (R&D Systems, Catalog # CLD001) per mL of culture media. CD4⁺ T cells were maintained in a 5% CO₂ incubator at 37 °C for 5 days. An aliquot of the cell culture supernates was removed, assayed for TGF- β 1, and measured 77.7 pg/mL (untreated) and 471 pg/mL (treated).

Human PBMCs were seeded at 1×10^6 /mL and cultured in RPMI supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin and left untreated. An aliquot of the cell culture supernate was removed, assayed for TGF- β 1, and measured 2854 pg/mL.

Mouse EL-4 cells were cultured in DMEM High Glucose supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin. Cells were then treated with 10 ng/mL PMA and 10 μ g/mL PHA for 24 hours. An aliquot of the cell culture supernate was removed, assayed for TGF- β 1, and measured 2969 pg/mL.

Rat spleen was taken from a pregnant Sprague Dawley rat, homogenized, and cultured in DME with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin at 37 °C and 5% CO₂. Rat splenocytes were treated with 50 ng/mL recombinant rat IL-2 and 5 μ g/mL PHA for 3 days. An aliquot of the cell culture supernate was removed, assayed for TGF- β 1, and measured 2716 pg/mL.

Porcine PK-15 cells were cultured in MEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin. Cells were treated with 1 μ g/mL LPS for 72 hours. An aliquot of the cell culture supernate was removed, assayed for TGF- β 1, and measured 1631 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant TGF- β 1. This assay also recognizes human TGF- β 1.2.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range TGF- β 1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Activin A
Activin RIA
Activin RIIA
Activin RIB
Activin RIIB
Agrin
BMP-1.1
BMP-2
BMP-3
BMP-3b
BMP-4
BMP-5
BMP-6
BMP-7
BMP-8a
BMP-8b
BMP-10
BMP-15
BMPR-IA
BMPR-IB
BMPR-II
Follistatin₂₈₈
Follistatin₃₀₀
Follistatin₃₁₅
GDF-5
GDF-7
GDF-9
GDF-11
GDF-15
Inhibin A
Inhibin B
LAP (TGF- β 1)
TGF- α
TGF- β RI
TGF- β RIII
TGF- β 3

Recombinant mouse:

Activin RI
Activin RIIA
Activin RIB
Activin RIIB
BMP-3
BMP-3b
BMP-4
BMP-6
BMP-7
BMP-9
BMPR-IA
BMPR-IB
BMPR-II
Follistatin₂₈₈
Inhibin A
TGF- β RI
TGF- β RIII

Other recombinants:

rat Agrin
zebrafish BMP-2
amphibian TGF- β 5
chinese hamster TGF- β 1-LAP

Natural protein:

porcine TGF- β 2

SPECIFICITY CONTINUED

Cross-reactivity - Cross-reactivity was observed with the factors listed below.

Recombinant Protein	Assay Diluent	
	RD1-21	RD1-73
Human BMP-9	<1%	<1%
Mouse BMP-10	<1%	<1%
Human Latent TGF- β 1	<1%	<1%
Activated Human Latent TGF- β 1	15%	15%
Equine TGF- β 1	3%	<1%
Human TGF- β 1.2	14%	12%
Human TGF- β 2	13%	12%

Interference - Significant interference was observed with recombinant human TGF- β RII and recombinant mouse TGF- β RII.

Human or Mouse TGF- β RII (ng/mL)	TGF- β 1 (pg/mL)	% TGF- β 1 Detected in presence of Human TGF- β RII		% TGF- β 1 Detected in presence of Mouse TGF- β RII	
		RD1-21	RD1-73	RD1-21	RD1-73
0	500	100%	100%	100%	100%
1.56	500	98%	99%	90%	93%
3.13	500	93%	95%	85%	88%
6.25	500	82%	86%	77%	79%
12.5	500	63%	66%	69%	67%
25	500	42%	37%	60%	55%
50	500	28%	18%	52%	40%

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

12									
11									
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NOTES

NOTES

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