Quantikine® ELISA

Mouse/Rat/Canine/Porcine TGF-β2 Immunoassay

Catalog Number MB200

For the quantitative determination of mouse, rat, canine, or porcine Transforming Growth Factor beta 2 (TGF-β2) concentrations in cell culture supernates, cell lysates, serum, and plasma.

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

TABLE OF CONTENTS

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INTRODUCTION

TGF-β2 (Transforming Growth Factor beta 2) is one of three closely related mammalian members of the large TGF-β superfamily that share a characteristic cysteine knot structure (1-7). TGF-β1, 2 and 3 are encoded by separate genes, but are often called isoforms. They are highly pleiotropic cytokines that are proposed to act as cellular switches regulating processes such as immune function, proliferation and epithelial-mesenchymal transition (4-8). Mammalian TGF-β2 is secreted as a 395 amino acid (aa) proprotein that is processed by a furin-like convertase to generate an N-terminal latency-associated peptide (LAP, ~232 aa) and a C-terminal mature TGF-β2 (~112 aa) that remain associated via hydrogen bonding (9-13). Serine proteases such as plasmin, matrix metalloproteases, or thrombospondin-1, along with cofactors such as certain integrins, can dissociate LAP and release active TGF-β2 (11-14). In many types of cells, latent TGF-β binding protein (LTBP) is covalently linked to the LAP homodimer prior to secretion. LTBP creates a large latent complex that is secreted, but may bind and localize to the extracellular matrix (10, 11). For TGF-β isoforms, the latency components act as natural antagonists of TGF-β activity, target TGF-β to distinct tissues, and maintain a reservoir of TGF-β (1). Mature mouse and rat TGF-β2 share 100% aa sequence identity, and share 97% aa identity with human, porcine, canine, equine and bovine TGF-β2.

TGF-β2 signaling begins with binding to a complex of the accessory receptor betaglycan (also known as TGF-β RIII) and the TGF-β RII type II ser/thr kinase receptor (15). In contrast, TGF-β1 and 3 have higher affinity for TGF-β RII and do not require betaglycan (15). TGF-β RII then phosphorylates and activates another ser/thr kinase receptor, TGF-β RI (also called activin receptor-like kinase (ALK) -5), or alternatively, ALK-1. The whole complex phosphorylates and activates Smad proteins that regulate transcription (1, 6, 15, 16). Differences in structure of the prodomains and mature sequences of TGF-β isoforms, and use of Smad-independent signaling pathways, allow for disparate actions observed in response to different TGF-β isoforms and contexts (1-3, 12, 13, 15, 16).

Although many functions are overlapping, each TGF-β isoform has some non-redundant functions. TGF-β2 plays a non-redundant role in human and mouse developmental heart valve remodeling, and mice with targeted deletions of TGF-β2 show defects in development of the cardiac system as well as lung, craniofacial, limb, eye, ear and urogenital systems (2, 7, 17, 28). TGF-β2 plays a unique positive regulatory role in hematopoiesis by enhancing Flt-3 signaling in hematopoietic progenitors (19). In humans, TGF-β isoforms, especially TGF-β2, are identified as key factors in the progression of malignant glioma, gastric and ovarian cancer (8, 20-22). TGF-β isoforms, particularly TGF-β2, suppress macrophage cytokine production and mucosal inflammatory responses in the developing intestine (23). In turn, macrophage LRP-1 can downregulate expression of TGF-β2 during vascular remodeling, which suppresses neo-intima formation (24).

The Quantikine® Mouse/Rat/Canine/Porcine TGF-β2 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure TGF-β2 in mouse, rat, canine, or porcine cell culture supernates, cell lysates, serum, and plasma. It contains CHO cell-expressed recombinant mouse mature TGF-β2 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant mouse TGF-β2. Results obtained using natural mouse, rat, canine, or porcine TGF-β2 showed dose response curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse/rat/canine/porcine TGF-β2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat/canine/porcine TGF-β2 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any TGF-β2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat/canine/porcine TGF-β2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of TGF-β2 bound in the initial step. The sample values are then read off the standard curve.

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.
- 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.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse/Rat/ Canine/Porcine TGF-β2 Microplate	894585	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for TGF-β2.	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.*
Mouse/Rat/ Canine/Porcine TGF-β2 Conjugate	894586	12 mL of a polyclonal antibody specific for TGF-β2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat/ Canine/Porcine TGF-β2 Standard	894587	Recombinant mouse TGF-β2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse/Rat/ Canine/Porcine TGF-β2 Control	894588	Recombinant mouse TGF-β2 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1-98	895596	11 mL of a buffered protein solution with preservatives.	
Calibrator Diluent RD5P	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and activation of samples.

If using cell lysate samples, the following are also required:

- Cell Lysis Buffer 1 (R&D Systems®, Catalog # 890713).
- PBS

ADDITIONAL REAGENTS REQUIRED

For sample activation:

- 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)

PRECAUTIONS

TGF-β2 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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 MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

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

Cell Culture Supernates - Remove particulates by centrifugation. Assay 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 TGF-β2. For best results, do not use animal serum for growth of cell cultures when assaying for TGF-β2 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-β2.*

Cell Lysates - Prior to assay, cells must be lysed according to the directions in the Sample Values section.

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 immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay 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.*

ACTIVATION REAGENT PREPARATION

To activate latent TGF-β2 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 or use of these reagents. Refer to the appropriate MSDS prior to 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.

SAMPLE ACTIVATION PROCEDURE

To activate TGF-β2 to immunoreactive TGF-β2 detectable by the Quantikine TGF-β2 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 controls. *The kit standards and controls contain mature recombinant TGF-β2. Use the chart below for volumes of 1N HCl, 1.2 N NaOH/0.5 M HEPES, and Calibrator Diluent RD5P (diluted 1:5)* used for specific sample types.*

1. Add 1N HCl to sample. Mix well. Incubate for 10 minutes at room temperature.
2. Add 1.2 N NaOH/0.5 M HEPES. Mix well.
3. Add Calibrator Diluent RD5P (diluted 1:5)*. Mix well and assay within 2 hours.

Note: *The concentration read off of the standard curve must be multiplied by the appropriate dilution factor.*

SAMPLE PREPARATION

Some samples require an additional dilution after sample activation.

Sample Type	Sample (µL)	1 N HCl (µL)	1.2 N NaOH/ 0.5 M HEPES (µL)	Calibrator Diluent RD5P (1:5) (µL)	Final Dilution Factor
Cell culture supernates & cell lysates	100	50	50	0	1:2
Mouse/Rat/Canine serum & plasma	40	20	20	80	1:4
Porcine serum & plasma	20	10	10	760	1:40

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse/Rat/Canine/Porcine TGF-β2 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

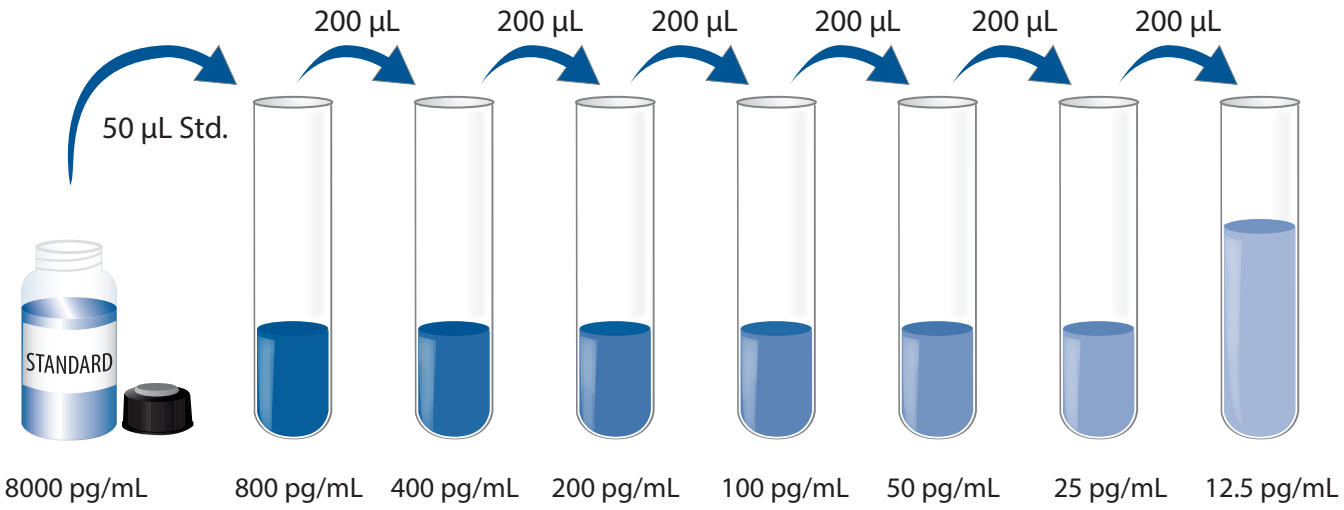
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Mouse/Rat/Canine/Porcine TGF-β2 Standard - Refer to the vial label for reconstitution volume. Reconstitute the Mouse/Rat/Canine/Porcine TGF-β2 Standard with deionized or distilled water. This reconstitution produces a stock solution of 8000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:5) into the 800 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube gently but thoroughly before the next transfer. The 800 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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, control, and samples be assayed in duplicate.

Note: *TGF-β2 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.*

1. Prepare all reagents, standard dilutions, control, and 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 µL of Assay Diluent RD1-98 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. 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 µL) 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 µL of Mouse/Rat/Canine/Porcine TGF-β2 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL 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 require activation and dilution. See Sample Activation and Sample Preparation sections.

CALCULATION OF RESULTS

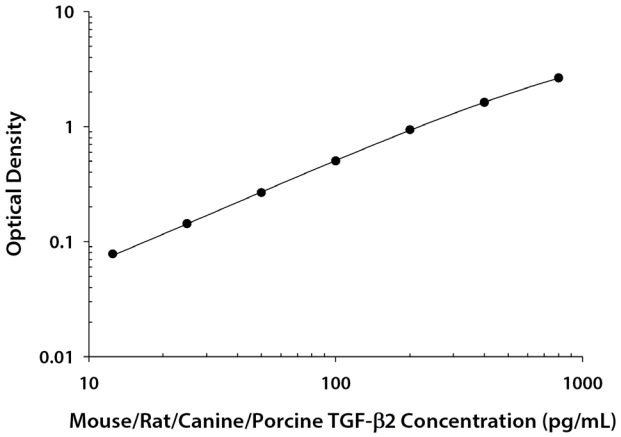
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse/rat/canine/porcine TGF-β2 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 activated and diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.035 0.036	0.036	—
12.5	0.109 0.118	0.114	0.078
25	0.176 0.181	0.179	0.143
50	0.295 0.309	0.302	0.266
100	0.533 0.543	0.538	0.502
200	0.952 0.993	0.973	0.937
400	1.618 1.694	1.656	1.620
800	2.663 2.688	2.676	2.640

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 twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	29.0	102	323	29.9	115	337
Standard deviation	2.43	4.97	21.0	2.80	8.20	30.7
CV (%)	8.4	4.9	6.5	9.4	7.2	9.1

RECOVERY

The recovery of mouse/rat/canine/porcine TGF-β2 spiked to levels throughout the range of the assay in various matrices was evaluated.

Mouse Samples	Average % Recovery	Range
Cell culture samples* (n=4)	92	80-102%
Cell lysates* (n=2)	94	86-98%
Serum* (n=4)	98	85-114%
EDTA plasma* (n=4)	84	78-94%
Heparin plasma* (n=4)	82	74-92%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of mouse/rat/canine/porcine TGF-β2 in each matrix were diluted with calibrator diluent and assayed.

Mouse Samples		Cell culture samples* (n=4)	Cell lysates* (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	99	100	97	108	108
	Range (%)	94-104	100-100	88-104	105-113	104-110
1:4	Average % of Expected	101	104	100	111	106
	Range (%)	93-106	102-106	95-104	108-113	100-109
1:8	Average % of Expected	105	102	103	116	105
	Range (%)	98-111	101-103	98-109	112-118	100-113
1:16	Average % of Expected	109	105	106	109	102
	Range (%)	101-118	101-108	101-114	102-118	99-108

*Samples were prepared as directed in the Sample Preparation section.

Note: *Rat/canine/porcine samples were evaluated and no significant difference in linearity or recovery was observed from the data above.*

SENSITIVITY

Twenty-seven assays were evaluated and the minimum detectable dose (MDD) of mouse/rat/canine/porcine TGF-β2 ranged from 0.789-4.26 pg/mL. The mean MDD was 1.66 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 a highly purified CHO cell-expressed recombinant mouse mature TGF-β2 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse/rat/canine/porcine TGF-β2 in this assay.

Mouse Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	157	117-211	26.5
EDTA plasma (n=5)	147	117-173	24.9

Rat Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	163	53-282	77.8
Heparin plasma (n=5)	257	124-342	87.6

Canine Samples	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=10)	71.5	60	ND-138
EDTA plasma (n=5)	103	40	ND-120
Heparin plasma (n=5)	65.7	60	ND-69.2

Porcine Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=4)	11,465	9000-14,160	2212

	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Mouse heparin plasma (n=5)	139	80	ND-169
Rat EDTA plasma (n=5)	158	60	ND-269

ND=Non-detectable

Cell Culture Supernates - Mouse lungs were rinsed with PBS and homogenized with a tissue homogenizer. Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 3 days. An aliquot of the cell culture supernate was removed, activated, assayed for TGF-β2, and measured 67.8 pg/mL.

Cell Lysates - C2C12 mouse myoblast cells were cultured in DMEM supplemented with 10% fetal bovine serum for 6 days. Cell culture media was removed by centrifugation. 2.0 mL of Cell Lysis Buffer 1 was added to the cell pellet and allowed to incubate for 60 minutes with gentle agitation. Debris was then removed by centrifugation. An aliquot of the cell lysate was removed, activated, assayed for TGF-β2, and measured 42.4 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse/rat/canine/porcine TGF-β2.

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 mouse/rat/canine/porcine TGF-β2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
Activin A
Activin RIB
Activin RIIB
TGF-β1
TGF-β RI
TGF-β RII
TGF-β RIII

Recombinant human:
Activin RIA
Activin RIIA
BMP-2
BMP-4
Follistatin
Inhibin A
Inhibin B
Latent TGF-β2
LTBP-1
LTBP-2
LTBP-3
TGF-α

Recombinant amphibian:
TGF-β5

Recombinant human TGF-β3 cross-reacts approximately 0.2% in this assay.

Recombinant human TGF-β2 is detectable in this assay. For optimal measurement of human TGF-β2, use the Quantikine® Human TGF-β2 ELISA Kit, Catalog # DB250.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.