Quantikine® ELISA

Mouse/Rat/Porcine/Canine TGF-β1 Immunoassay

Catalog Number MB100B
SMB100B
PMB100B

For the quantitative determination of activated mouse/rat/porcine/canine Transforming Growth Factor beta 1 (TGF-β1) concentrations in cell culture supernates, 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.

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INTRODUCTION

Transforming growth factor beta (TGF-β) proteins (including the three closely related mammalian isoforms TGF-β1, -2 and -3) are pleiotropic cytokines that regulate extracellular matrix production, wound healing, immune functions, cell proliferation and differentiation. They belong to the large TGF-β superfamily, which also includes the activins/inhibins/MIS, bone morphogenetic proteins (BMPs), growth/differentiation factors (GDFs), Lefty1 and 2, and the distantly related GDNF family of neurotrophic factors. All family members show a characteristic cysteine-knot that is formed from multiple intrachain disulfide bonds (1-3).

The mouse, rat, porcine and canine TGF-β1 cDNAs encode a 390 amino acid (aa) residue precursor that contains a 29 aa signal peptide and a 361 aa pro-protein. The pro-protein for each species is proteolytically processed via a furin-like convertase to generate an N-terminal 249 aa latency-associated peptide (LAP), and a C-terminal 112 aa mature TGF-β1 (4-9). Both LAP and mature TGF-β1 exist as disulfide-linked homodimers. After proteolytic cleavage and secretion, the two homodimers remain non-covalently associated as the small latent TGF-β1 complex. In most cell types, this complex is also covalently linked via LAP to a latent TGF-β binding protein (LTBP). This creates a secreted, large latent complex. The TGF-β1 present in either the small or the large latent complex is not available for TGF-β receptor binding and activation, and is, therefore, latent. Whereas LAP is both necessary and sufficient to confer latency to TGF-β1, LTBP facilitates the proper folding and secretion of the small latent complex. LTBP is also a structural component of the extracellular matrix and directs the localization of the latent complex to the extracellular matrix (10-15). To date, four LTBPs that share multiple EGF-like, LTBP- and fibrillin-specific domains have been cloned. Three of the four LTBPs (#1, 3, and 4) have been shown to bind the small latent complexes of all TGF-β isoforms (16). Activation of the latent TGF-β complex is an important step that regulates TGF-β function *in vivo*. Multiple activation mechanisms have been identified. These involve protease-dependent (plasmin and matrix metalloprotease) and protease-independent (binding of LAP to thrombospondin 1 or a subset of integrins) pathways (5, 15, 17-21). Mature mouse TGF-β1 shows 100% aa sequence identity to mature rat TGF-β1 (6, 7), and both rat and mouse TGF-β1 show 99% aa sequence identity with mature porcine, human, and canine TGF-β1 (4, 9, 22). Mature porcine TGF-β1 shows absolute identity to both mature human and canine TGF-β1.

The signalling high-affinity receptor for TGF-β1 is a heteromeric complex consisting of a type I (TGF-β RI) and a type II (TGF-β RII) transmembrane serine/threonine kinase receptor. TGF-β RII is a constitutively active kinase. Upon binding TGF-β1, it phosphorylates and activates the TGF-β RI. In turn, TGF-β RI phosphorylates and activates Smad proteins that regulate transcription. TGF-β RI, alternatively named activin receptor-like kinase (ALK-5), is present in almost all cell types. Two other type I receptors, ALK-1 and ALK-2 have also been implicated as alternative partners for TGF-β RII in the TGF-β signalling receptor complex. Besides the type I and type II receptors, accessory receptors including the type III receptor, TGF-β RIII (also known as betaglycan), and endoglin, which modulate TGF-β responses, have been identified (2, 23-26).

The Quantikine Mouse/Rat/Porcine/Canine TGF-β1 Immunoassay is a 4.5 hour solid phase ELISA designed to measure TGF-β1 in cell culture supernates, serum, and plasma. It contains recombinant TGF-β1 expressed by CHO cells and has been shown to quantitate the recombinant factor accurately. Results obtained using natural TGF-β1 showed linear curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that this kit will provide accurate quantitation for both recombinant and natural TGF-β1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TGF-β1 has been pre-coated onto a microplate. Standards, Control and samples are pipetted into the wells and any TGF-β1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TGF-β1 is added to the wells to sandwich the TGF-β1 immobilized during the first incubation. 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-β1 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 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 standard 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 this 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.

- For best results, pipette reagents and samples into the center of each well.

- It is recommended that the samples be pipetted within 15 minutes.

- 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 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # MB100B	CATALOG # SMB100B	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 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. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a new Standard and Control for each assay.
TGF-β1 Control	891127	2 vials	12 vials	Recombinant TGF-β1 in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the vial label.	
TGF-β1 Conjugate	893003	1 vial	6 vials	12 mL/vial of a polyclonal antibody against 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. *For cell culture supernate samples.*	
Assay Diluent RD1-73	895541	1 vial	3 vials	12 mL/vial of a buffered base with preservatives. *For serum/plasma samples.*	
Calibrator Diluent RD5-53	895587	1 vial	3 vials	21 mL/vial of a concentrated buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use. Use diluted 1:4 in this assay.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
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.

MB100B contains sufficient materials to run ELISAs on one 96 well plate.
SMB100B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PMB100B). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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)

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.

PRECAUTIONS

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

Some components in this kit contain ProClin® 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. Please refer to the MSDS on our website prior to use.

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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-β1. For best results, do not use animal serum for growth of cell cultures when assaying for TGF-β1 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-β1.*

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.

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.

*TGF-β1 is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of TGF-β1, 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 blood. This will cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation. The recommended plasma collection protocol is designed to minimize platelet degranulation. However, since even the best methods for plasma collection may result in some platelet degranulation on occasion, it is recommended that markers for platelet degranulation be determined in samples containing elevated TGF-β1 levels.

ACTIVATION REAGENT PREPARATION

To activate latent TGF-β1 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 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 control.* *The kit standards and control contain active recombinant TGF-β1.*

Cell Culture Supernates	Serum/Plasma
To 100 µL of cell culture supernate, add 20 µL of 1 N HCl.	To 40 µL of serum/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.
Assay immediately.	Prior to the assay, dilute the activated sample with Calibrator Diluent. *See below for suggested dilutions.*
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.

Mouse and rat serum/platelet-poor plasma* samples require a 60-fold dilution in Calibrator Diluent RD5-53 (diluted 1:4)[†]. A suggested 60-fold dilution is 10 µL of activated sample + 590 µL of Calibrator Diluent RD5-53 (diluted 1:4) (final dilution factor of sample is 90).

Porcine serum samples require a 15-fold dilution in Calibrator Diluent RD5-53 (diluted 1:4). A suggested 15-fold dilution is 10 µL of activated sample + 140 µL of Calibrator Diluent RD5-53 (diluted 1:4) (final dilution factor of sample is 22.5).

Porcine platelet-poor plasma* samples require a 5-fold dilution in Calibrator Diluent RD5-53 (diluted 1:4). A suggested 5-fold dilution is 40 µL of activated sample + 160 µL of Calibrator Diluent RD5-53 (diluted 1:4) (final dilution factor of sample is 7.5).

Canine serum and platelet-poor plasma* samples require a 40-fold dilution in Calibrator Diluent RD5-53 (diluted 1:4). A suggested 40-fold dilution is 10 µL of activated sample + 390 µL of Calibrator Diluent RD5-53 (diluted 1:4) (final dilution factor of sample is 60).

*May require different dilutions depending on the extent of platelet contamination.
[†]See Regeant Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

TGF-β1 Control - Reconstitute the Control with 1.0 mL deionized or distilled water. Assay the Control undiluted. Do not activate.
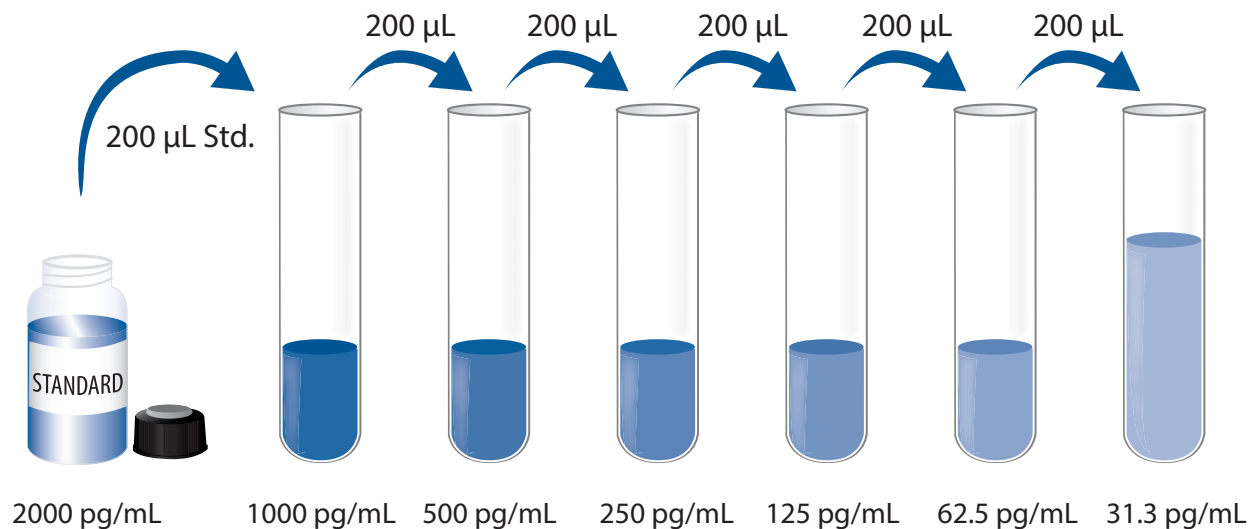
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5-53 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-53 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-53 (1X). *May contain a precipitate. Mix well before and during use.*

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.

TGF-β1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the TGF-β1 Standard with Calibrator Diluent RD5-53 (diluted 1:4). 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 μL of Calibrator Diluent RD5-53 (diluted 1:4) into each tube. Use the standard stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted TGF-β1 Standard serves as the high standard (2000 pg/mL). Calibrator Diluent RD5-53 (diluted 1:4) serves as the zero standard (0 pg/mL).

| 200 μL Std. | 200 μL | 200 μL | 200 μL | 200 μL | 200 μL |

| 2000 pg/mL | 1000 pg/mL | 500 pg/mL | 250 pg/mL | 125 pg/mL | 62.5 pg/mL | 31.3 pg/mL |

ASSAY PROCEDURE

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

1. Prepare all reagents, standard dilutions, Control, 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 µL of Assay Diluent RD1-21 (*for cell culture supernate samples*) or Assay Diluent RD1-73 (*for serum/plasma samples*) to each well.

4. Add 50 µL 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 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 TGF-β1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.

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. **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 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-β1 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 ASSAY
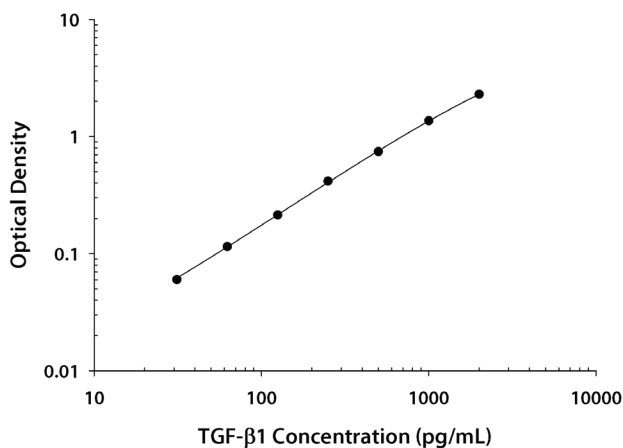
(pg/mL)	O.D.	Average	Corrected
0	0.050 0.052	0.051	—
31.3	0.107 0.114	0.111	0.060
62.5	0.165 0.167	0.166	0.115
125	0.262 0.267	0.265	0.214
250	0.459 0.474	0.467	0.416
500	0.781 0.809	0.795	0.744
1000	1.372 1.390	1.381	1.330
2000	2.322 2.368	2.345	2.294

SERUM/PLASMA ASSAY
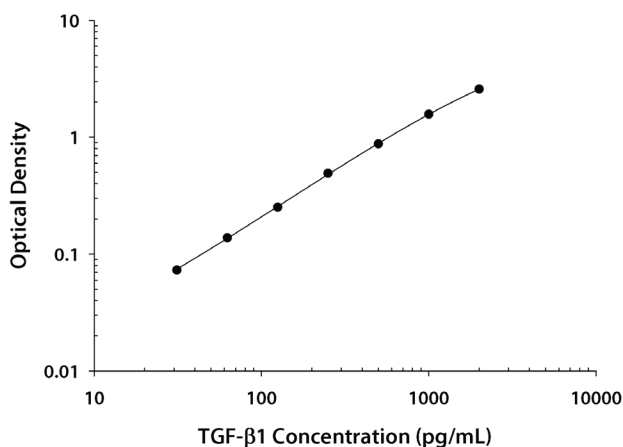
(pg/mL)	O.D.	Average	Corrected
0	0.053 0.053	0.053	—
31.3	0.124 0.128	0.126	0.073
62.5	0.189 0.193	0.191	0.138
125	0.303 0.307	0.305	0.252
250	0.542 0.545	0.544	0.491
500	0.924 0.934	0.929	0.876
1000	1.611 1.638	1.625	1.572
2000	2.624 2.630	2.627	2.574

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 ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	22	21	22
Mean (pg/mL)	88	198	816	97	209	826
Standard deviation	3.0	4.9	19.6	8.1	17.3	47.0
CV (%)	3.4	2.5	2.4	8.4	8.3	5.7

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	28	37	30
Mean (pg/mL)	78	180	695	79	185	730
Standard deviation	2.3	3.4	17.3	7.2	17.2	46.8
CV (%)	2.9	1.9	2.5	9.1	9.3	6.4

RECOVERY

The recovery of TGF-β1 spiked to levels throughout the range of the assay followed by activation was evaluated.

Sample Type	Average % Recovery	Range
Mouse cell culture supernates (n=4)	105	83-114%
Rat cell culture supernates (n=3)	112	93-125%
Porcine cell culture supernates (n=2)	109	102-118%

LINEARITY

To assess linearity of the assay, the activated samples containing and/or spiked with high concentrations of TGF-β1, were diluted with Calibrator Diluent and then assayed.

| 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	99	101	93	98
	Range (%)	95-103	99-103	83-99	94-101
1:4	Average % of Expected	99	101	92	95
	Range (%)	92-104	100-104	88-96	92-98
1:8	Average % of Expected	97	99	91	98
	Range (%)	92-99	96-102	85-96	92-108
1:16	Average % of Expected	93	102	91	92
	Range (%)	80-103	99-105	89-95	91-94

| 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	102	98	98	98
	Range (%)	101-102	95-101	93-104	90-103
1:4	Average % of Expected	104	100	94	92
	Range (%)	102-107	97-102	89-103	85-95
1:8	Average % of Expected	106	100	93	93
	Range (%)	102-111	99-102	87-101	83-103
1:16	Average % of Expected	108	99	93	90
	Range (%)	102-115	93-105	91-95	84-99

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

Porcine Samples		Cell culture supernates (n=2)	Serum* (n=4)	Platelet-poor	
				EDTA plasma* (n=5)	Heparin plasma* (n=4)
1:2	Average % of Expected	100	102	107	110
	Range (%)	98-102	99-105	102-110	105-113
1:4	Average % of Expected	102	104	115	107
	Range (%)	99-104	102-105	109-124	95-117
1:8	Average % of Expected	114	105	———	114
	Range (%)	112-116	102-109	———	102-125
1:16	Average % of Expected	107	101	———	121
	Range (%)	95-119	97-108	———	112-127

Canine Samples		Serum* (n=4)	Platelet-poor	
			EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	100	103	101
	Range (%)	93-105	96-107	97-104
1:4	Average % of Expected	102	106	104
	Range (%)	93-109	100-112	97-110
1:8	Average % of Expected	103	107	104
	Range (%)	94-115	101-114	86-115
1:16	Average % of Expected	95	96	———
	Range (%)	83-110	———	———

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

SENSITIVITY

Ten assays were evaluated and the minimum detectable dose (MDD) of TGF-β1 ranged from 1.7-15.4 pg/mL. The mean MDD was 4.6 pg/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant human TGF-β1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of TGF-β1 in this assay.

Mouse Samples	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=20)	99	75-128	16
Platelet-poor EDTA plasma (n=10)	102	51-175	45
Platelet-poor heparin plasma (n=10)	60	18-95	23

Rat Samples	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=20)	52	30-71	11
Platelet-poor EDTA plasma (n=10)	29	8-69	24
Platelet-poor heparin plasma (n=10)	79	25-124	30

Porcine Samples	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=15)	15	9-22	4
Platelet-poor EDTA plasma (n=10)	1.0	0.9-1.6	0.4
Platelet-poor heparin plasma (n=5)	6	3.4-12	3.5

Canine Samples	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=10)	53	38-72	11
Platelet-poor EDTA plasma (n=5)	7	4-9	2.1
Platelet-poor heparin plasma (n=5)	20	13-30	8.4

Cell Culture Supernates:

Cell culture supernate samples were evaluated for the presence of TGF-β1 in this assay.

Sample	Mean (pg/mL)
DMEM + 10% fetal calf serum	933
RPMI + 5% fetal calf serum	493
Mouse EL4/IL-2*	1522
PK15*	1414
Rat splenocytes*	1215

*Values have not been corrected for TGF-β1 levels in the fetal calf serum.

EL-4 mouse lymphoblast cells (1 x 10^6 cells/mL) were cultured for 3 days in DMEM supplemented with 10% fetal calf serum and stimulated with 10 µg/mL PHA and 10 ng/mL PMA. An aliquot of the cell culture supernate was removed and assayed for levels of TGF-β1.

PK-15 porcine kidney epithelial cells (1 x 10^6 cells/mL) were grown to confluency in RPMI supplemented with 10% fetal calf serum and stimulated with 100 ng/mL LPS. Samples were cultured for 24 hours. An aliquot of the cell culture supernate was removed and assayed for levels of TGF-β1.

Rat splenocyte cells (1 x 10^6 cells/mL) were cultured for 3 days in DMEM supplemented with 10% fetal calf serum. An aliquot of the cell culture supernate was removed and assayed for levels of TGF-β1.

SPECIFICITY

This assay recognizes natural and recombinant TGF-β1. This assay also recognizes human TGF-β1.2.

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-β1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Activin A	BMPR-IB
Activin RIA	BMPR-II
Activin RIIA	Follistatin$_{288}$
Activin RIIB	Follistatin$_{300}$
BMP-2	Follistatin$_{315}$
BMP-3	Inhibin A
BMP-3b	Inhibin B
BMP-4	LAP (TGF-β1)
BMP-5	TGF-α
BMP-6	TGF-β RI
BMP-8b	TGF-β RII
BMP-10	TGF-β RIII
BMP-15	TGF-β2
BMPR-IA	TGF-β3

Recombinant mouse:

BMP-3b
BMPR-IA
BMPR-IB
TGF-β RI

Other recombinants:

rat Agrin
zebrafish BMP-2a
amphibian TGF-β5

Natural protein:

porcine TGF-β2

Cross-reactivity - This kit detects Latent TGF-β1 complex after acid treatment.

Interference - Significant interference was observed with rmTGF-β RII.

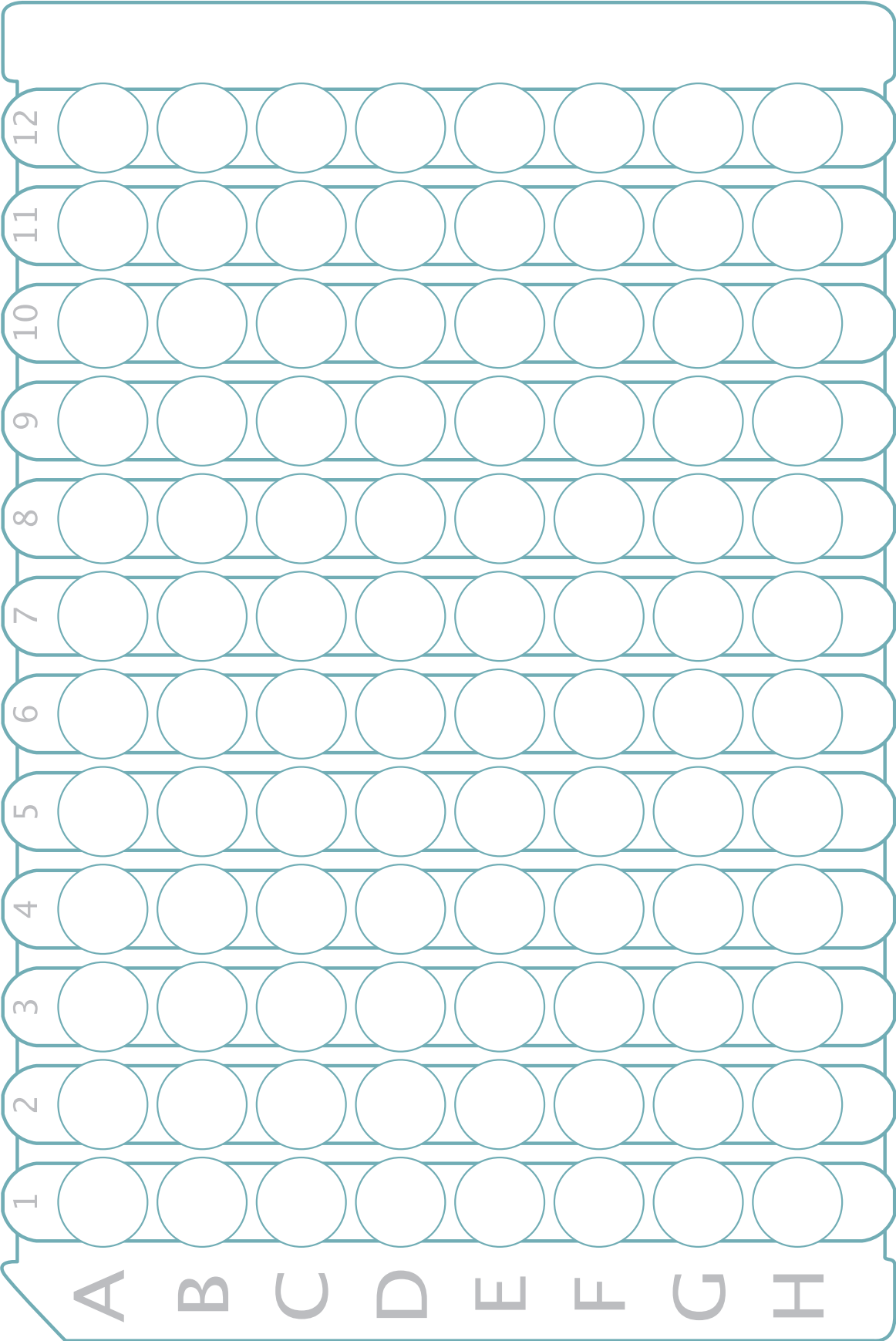| Mouse TGF-β RII (ng/mL)	TGF-β1 (pg/mL)	% TGF-β1 Detected	
		Assay Diluent RD1-21	Assay Diluent RD1-73
0	700	100%	100%
1.56	700	91%	94%
3.12	700	84%	83%
6.25	700	68%	67%
12.5	700	45%	40%
25	700	25%	20%
50	700	19%	13%

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

For research use only. Not for use in diagnostic procedures.

NOTES

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