



PRODUCT INFORMATION & MANUAL

Mouse/Rat/Porcine/Canine TGF- β 1 Valukine™ ELISA

Catalog Number: VAL611

For the quantitative determination of natural and recombinant
Mouse/Rat/Porcine/Canine TGF- β 1 concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202407.9

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I. BACKGROUND

Transforming growth factor beta (TGF- β) proteins (including the three closely related mammalian isoforms TGF- β 1, -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- β 1 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- β 1 (4-9). Both LAP and mature TGF- β 1 exist as disulfide-linked homodimers. After proteolytic cleavage and secretion, the two homodimers remain non-covalently associated as the small latent TGF- β 1 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- β 1 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- β 1, 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- β 1 shows 100% aa sequence identity to mature rat TGF- β 1 (6, 7), and both rat and mouse TGF- β 1 show 99% aa sequence identity with mature porcine, human, and

canine TGF- β 1 (4, 9, 22). Mature porcine TGF- β 1 shows absolute identity to both mature human and canine TGF- β 1.

The signalling high-affinity receptor for TGF- β 1 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- β 1, 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).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for TGF- β 1 has been pre-coated onto a microplate. Standards 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 antibody specific for TGF- β 1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate solution (Chromogenic agent) 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.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernate and mouse/rat/porcine/canine serum.
- 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, dilute the samples with Calibrator Diluent (1 $\times$) and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Two samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision		Inter-assay Precision		
Sample	1	2	1	2	3
Mean (pg/mL)	88	198	816	97	209
Standard Deviation	3	4.9	19.6	8.1	17.3
CV%	3.4	2.5	2.4	8.4	8.3

B. RECOVERY

The recovery of TGF- β 1 spiked to different levels throughout the range of the assay in activated cell culture supernates was evaluated. The recovery ranged from 83 to 114% with an average of 105%.

The recovery of TGF- β 1 spiked to different levels throughout the range of the assay in activated serum was evaluated. The recovery ranged from 74.5 to 90.2% with an average of 78.9%.

C. SENSITIVITY

The minimum detectable dose (MDD) of TGF- β 1 is typically less than 1.7-15.4 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.

D. CALIBRATION

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

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with

high concentrations of TGF- β 1 and diluted with Calibrator Diluent (1 $\times$) to produce samples with values within the dynamic range of the assay.

Dilution	Mouse		Rat		Porcine	
	Average% of Expected	Range (%)	Average% of Expected	Range (%)	Average% of Expected	Range (%)
1:2	99	95-103	102	101-102	100	98-102
1:4	99	92-104	104	102-107	102	99-104
1:8	97	92-99	106	102-111	114	112-116
1:16	93	80-103	108	102-115	107	95-119

F. SAMPLE VALUES

Cell Culture Supernates - Cell culture supernate samples were evaluated for the presence of TGF- β 1 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- β 1 levels in the fetal calf serum.

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

PK-15 porcine kidney epithelial cells (1×10^6 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- β 1.

Rat splenocyte cells (1×10^6 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- β 1.

Serum - Four mouse serum samples were evaluated for the presence of TGF- β 1 in this assay. All samples measured ranged from 94.5 to 120 ng/mL with an average of 106 ng/mL.

G. SPECIFICITY

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

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent (1 $\times$) 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		Recombinant Mouse
Activin A	BMPR-IB	BMP-3b
Activin RIA	BMPR-II	TGF- β RI
Activin RIIA	Follistatin288	BMPR-IA
Activin RIIB	Follistatin300	BMPR-IB
BMP-2	Follistatin315	
BMP-3	Inhibin A	Other Recombinant
BMP-3b	Inhibin B	Rat Agrin
BMP-4	LAP (TGF- β 1)	Zebrafish BMP-2a
BMP-5	TGF- α	Amphibian TGF- β 5
BMP-6	TGF- β RI	
BMP-8b	TGF- β RII	Nature protein
BMP-10	TGF- β RIII	Porcine TGF- β 2
BMP-15	TGF- β 2	
BMPR-IA	TGF- β 3	

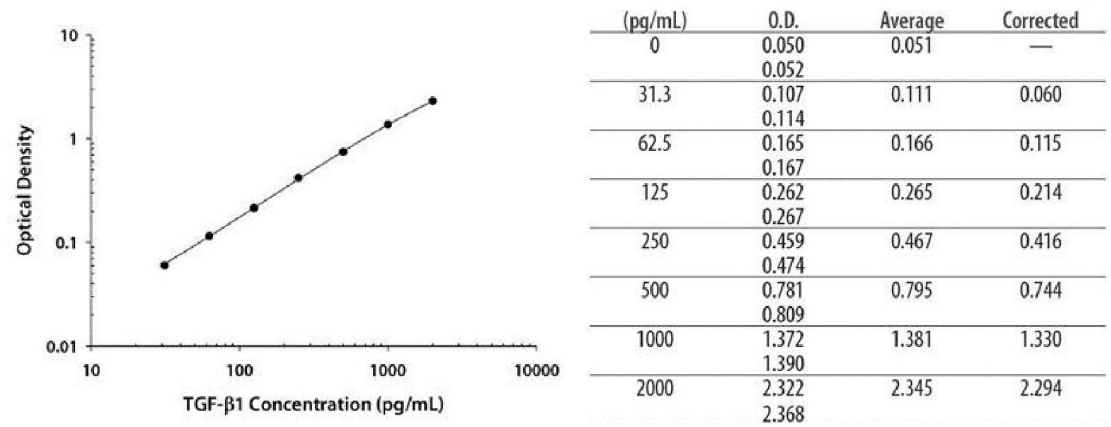
Cross-reactivity - This kit detects Latent TGF- β 1 complex after acid treatment.

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

IV. EXPERIMENT

EXAMPLE STANDARD

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



V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
TGF- β 1 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against TGF- β 1	1 plate
TGF- β 1 Conjugate	Solution of antibody against TGF- β 1 conjugated to horseradish peroxidase with preservatives	1 vial
TGF- β 1 Standard	Recombinant TGF- β 1 in a buffered protein base with preservatives; lyophilized. Refer to the vial label for reconstitution volume	2 vials
Calibrator Diluent (2 $\times$)/RD6-11	A 2 $\times$ concentrated buffered protein base used to dilute standard and samples	2 vials
Assay Diluent/RD1-21	A buffered protein base with preservatives	1 vial
Wash Buffer Concentrate (25 $\times$)	A 25 $\times$ concentrated solution of buffered surfactant with preservatives	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	1 vial
Stop Solution	Diluted hydrochloric acid	1 vial
Plate Sealers	Adhesive strips	3 strips

B. STORAGE

Unopened Kit	Store at 2-8 °C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Wash Buffer (1×)	May be stored for up to 1 month at 2-8 °C.*
	Stop Solution	
	Conjugate	
	Assay Diluent/RD1-21	
	TMB Substrate	
	Standard	Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
	Calibrator Diluent (2×)/RD6-11	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.
	Microplate Wells	Return unused wells to the pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*

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

C. 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.
- 500 mL graduated cylinder.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE ACTIVATION PROCEDURE

To activate latent TGF- β 1 to immunoreactive TGF- β 1 detectable by the TGF- β 1 immunoassay, follow the activation procedure below. Assay samples is pH 7.2-7.6 after neutralization. **Use polypropylene test tubes.**

Cell Culture Supernates	Serum
To 100 μ L of cell culture supernate, add 20 μ L of 1 N HCl.	To 40 μ L serum, add 20 μ L 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 μ L of 1.2 N NaOH/0.5 M HEPES.	Neutralize the acidified sample by adding 20 μ L 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 (1×). See the following 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 factors*.

***Activated serum samples may be stored for up to 24 hours at 2-8 °C before use. Activated cell culture supernates samples must be assayed immediately after activation. Do not freeze activated samples.**

C. SAMPLE PREPARATION

Cell culture supernate samples recommend at least a 1.4-fold dilution prior to the assay. A suggested 1.4-fold dilution is activation of the sample. Optimal dilutions should be determined by the end user.

*Activated serum samples recommend at least a 60-fold dilution prior to the assay. For example, add 10 µL of serum into a tube with 90 µL Calibrator Diluent (1×) to prepare a 10-fold diluted sample. Mix through and then pipette 20 µL of prepared 10-fold diluted sample into a tube with 100 µL Calibrator Diluent (1×) to prepare a final 60-fold diluted sample. (The final dilution factor, 120.). Optimal dilutions should be determined by the end user.

D. REAGENT PREPARATION

Note: *Bring all reagents to room temperature before use.*

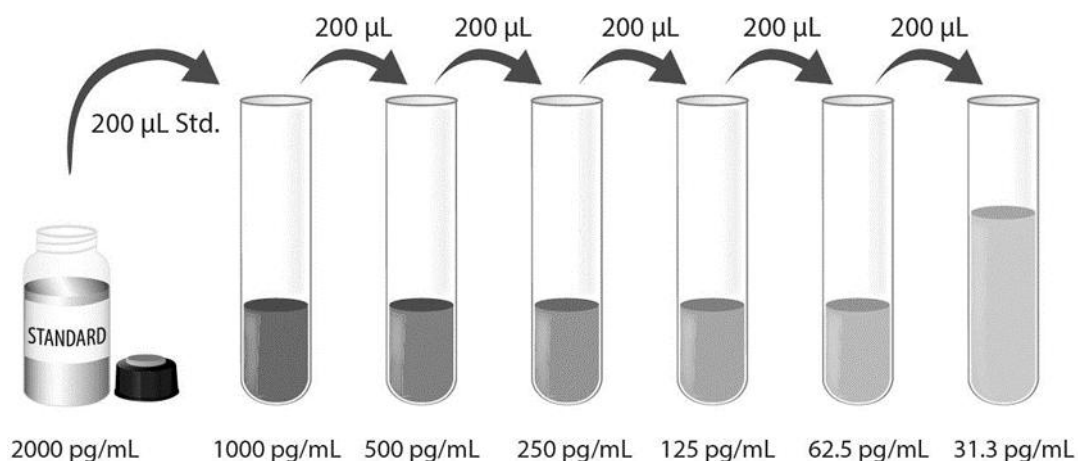
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

TGF-1 Standard - Refer to the vial label for reconstitution volume*. Reconstitute the TGF-β1 Standard with Calibrator Diluent (1×). This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

*If you have any question, please seek help from our Technical Support.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent (1×) 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 (1×) serves as the zero standard (0 pg/mL).



E. 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.
- TMB substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB substrate. 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 TMB substrate.

VII. ASSAY PROCEDURE

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

1. Prepare all reagents and working standards 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 to each well.
4. Add 50 μ L of Standard and sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature.** A plate layout is provided for a record of 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.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μ L of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. 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 10 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.

11. CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. 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.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

1								
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	A	B	C	D	E	F	G	H



产品信息及操作手册

小鼠/大鼠/猪/犬 TGF- β 1 Valukine™ ELISA 试剂盒

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I. 背景

转化生长因子 β (TGF- β) 蛋白 (包括三个密切相关的哺乳动物亚型TGF- β 1, -2和-3) 是调节细胞外基质产生、伤口愈合、免疫功能、细胞增殖和分化的多效性细胞因子。它们属于大TGF- β 超家族, 其中还包括激活素/抑制素/MIS、骨形态发生蛋白 (BMPs)、生长分化因子 (GDFs)、Lefty1和2和GDNF家族的亲缘关系较远的神经营养因子。所有的家庭成员表现出由多个链内二硫键形成典型的半胱氨酸结 (1-3)。

小鼠、大鼠、猪、犬 β TGF- β 1基因编码390个氨基酸 (AA) 残基的前体, 其中含有29个氨基酸的信号肽和361个氨基酸的前体蛋白。每个物种的前体蛋白经由通过弗林样转化酶蛋白水解处理, 生成N-末端249个氨基酸的latency-associated peptide (LAP), 和一个C末端112个氨基酸的成熟TGF- β 1 (4-9)。LAP和成熟的TGF- β 1都以二硫键连接的同源二聚体形式存在。经蛋白水解剪切和分泌后, 两个同源二聚体仍然以非共价结合形成小latent TGF- β 1复合物。在大多数细胞类型, 这种复合物也可通过LAP共价结合一个latent TGF- β 1结合蛋白 (LTBP)。这会产生一个分泌型的、巨大的latent复合物。存在于小的或大的latent复合物中的TGF- β 1, 不能用于TGF- β 受体的结合和激活, 因此是latent的。而LAP对于转化latent型到TGF- β 1是必要的和足够的, LTBP促进了小latent复合物的正确折叠和分泌。LTBP也是细胞外基质的结构成分, 它引导了latent复合物定位到细胞外基质 (10-15)。到目前为止, 四种共享有多个表皮生长因子样的、LTBP-和原纤维蛋白特异性的域的LTBPs已被克隆出来。四个LTBPs之中的三个 (1, 3, 4) 已被证明可结合所有TGF- β 异构体的小latent复合体 (16)。激活TGF- β 复合物是体内调节TGF- β 功能的重要步骤。已确定多种激活机制。这些涉及蛋白酶依赖性 (纤溶酶和基质金属蛋白酶) 和蛋白酶不依赖性 (LAP与血小板反应蛋白1或整合素一个亚型结合) 的通路 (5, 15, 17-21)。成熟小鼠TGF- β 1显示与成熟大鼠TGF- β 1的100%氨基酸序列同源性 (6, 7)。成熟大鼠和小鼠的TGF- β 1与成熟猪、人和犬的TGF- β 1显示99%氨基酸序列同源性 (4, 9, 22)。成熟猪TGF- β 1与成熟的人和犬TGF- β 1具有绝对的同源性。

TGF- β 1信号的高亲和力受体是由一个异源复合物组成的, 包括I型 (TGF- β RI) 和II型 (TGF- β RII) 跨膜丝氨酸/苏氨酸激酶受体。TGF- β RII是结构性活化激酶。一旦结合TGF- β 1, 它便磷酸化和激活TGF- β RI。接着, TGF- β RI磷酸化和激活了调节转录的Smad蛋白。

TGF- β RI, 或者叫激活素受体样激酶 (ALK-5), 存在于几乎所有类型的细胞中。在其它两个I型受体, ALK-1和ALK-2也是位于TGF- β 信号受体复合物中TGF- β RII的可供选择的合作伙伴。除了I和II型受体, 调节TGF- β 反应的辅助受体包括III型受体, TGF- β RIII (也称为 β 聚糖), 和内皮糖蛋白, 也已经确定 (2, 23-26)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗TGF- β 1抗体包被于微孔板上，样品和标准品中的TGF- β 1会与固定在微孔板上的抗体结合，游离的成分被洗去；加入辣根过氧化物酶标记的TGF- β 1抗体进行孵育，未结合的抗体被洗去；加入TMB底物溶液（显色剂），溶液颜色与结合的目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- 仅供科研使用，不可用于体外诊断；
- 该试剂盒适用于细胞培养上清样本和小鼠/大鼠/猪/犬血清；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释液（1×）稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的两个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板中分别检测20次，以确定板间精确度。

	板内精确度		板间精确度		
样本	1	2	1	2	3
平均值 (pg/mL)	88	198	816	97	209
标准差	3	4.9	19.6	8.1	17.3
CV%	3.4	2.5	2.4	8.4	8.3

B. 回收率

在活化的细胞上清样本中掺入检测范围内不同水平的TGF- β 1，测定其回收率。回收率范围在83-114%，平均回收率在105%。

在活化的血清样本中掺入检测范围内不同水平的TGF- β 1，测定其回收率。回收率范围在74.5-90.2%之间，平均回收率在78.9%。

C. 灵敏度

TGF- β 1的最低可测值1.7-15.4 pg/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对对应浓度。

D. 校正

此ELISA试剂盒经由R&D Systems®生产的CHO表达的高纯度重组TGF- β 1蛋白所校正。

E. 线性

不同的活化样本中掺入高浓度的TGF- β 1，然后用标准品稀释液（1 $\times$ ）将样本稀释到检测范围内，测定其线性。

稀释倍数	小鼠		大鼠		猪	
	平均值/期望值 (%)	范围 (%)	平均值/期望值 (%)	范围 (%)	平均值/期望值 (%)	范围 (%)
1:2	99	95-103	102	101-102	100	98-102
1:4	99	92-104	104	102-107	102	99-104
1:8	97	92-99	106	102-111	114	112-116
1:16	93	80-103	108	102-115	107	95-119

F. 样本预值

细胞上清样本 - 此方法测定细胞上清样品中TGF- β 1含量。

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

*测定值未校正血清中的 TGF- β 1 量。

按 1 $\times$ 10⁶ 细胞/mL 比例接种小鼠淋巴细胞系 EL-4 于 DMEM 培养基，其中含 10% FCS、10 μ g/mL PHA 和 10 ng/mL PMA，培养 3 天后，取上清检测 TGF- β 1。

按 1 $\times$ 10⁶ 细胞/mL 比例接种猪肾上皮细胞系 PK-15 于 RPMI 培养基，其中含 10% FCS 和 100ng/mL LPS，培养 24h 后，取上清检测 TGF- β 1。

按 1 $\times$ 10⁶ 细胞/mL 比例接种大鼠脾细胞于 DMEM 培养基，其中含 10% FCS，培养 3 天后，取上清检测 TGF- β 1。

血清样本 - 使用本试剂盒检测了4份小鼠血清样本中TGF- β 1的水平。所有样本的检测值在94.5-120 ng/mL之间，平均值为106 ng/mL。

G. 特异性

此ELISA法可检测天然及重组TGF- β 1蛋白，也可识别人TGF- β 1。将以下因子用标准品稀释液（1 $\times$ ）配制成50 ng/mL的浓度来检测交叉反应。将50 ng/mL的干扰因子掺入中间范围的TGF- β 1对照品中，来检测干扰。没有观察到明显的交叉反应或干扰。

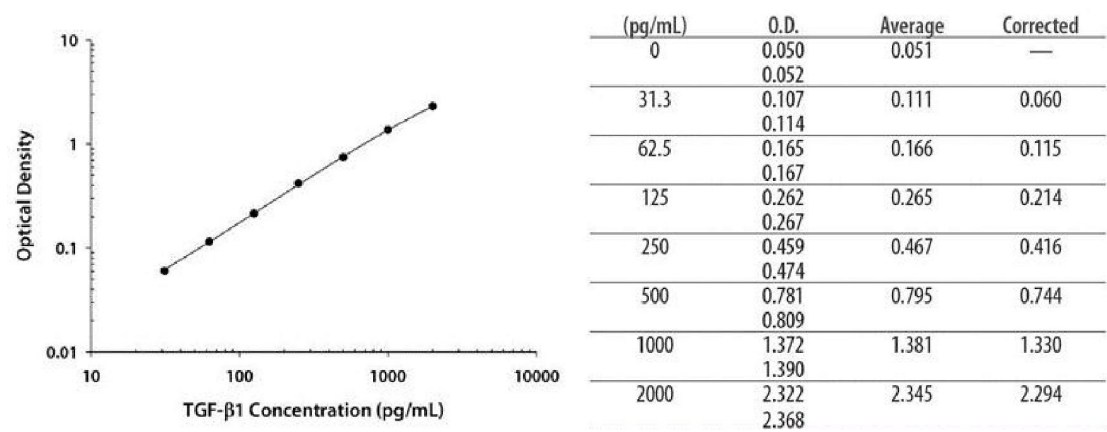
Recombinant Human		Recombinant Mouse
Activin A	BMPR-IB	BMP-3b
Activin RIA	BMPR-II	TGF- β RI
Activin RIIA	Follistatin288	BMPR-IA
Activin RIIB	Follistatin300	BMPR-IB
BMP-2	Follistatin315	
BMP-3	Inhibin A	Other Recombinant
BMP-3b	Inhibin B	Rat Agrin
BMP-4	LAP (TGF- β 1)	Zebrafish BMP-2a
BMP-5	TGF- α	Amphibian TGF- β 5
BMP-6	TGF- β RI	
BMP-8b	TGF- β RII	Nature protein
BMP-10	TGF- β RIII	Porcine TGF- β 2
BMP-15	TGF- β 2	
BMPR-IA	TGF- β 3	

交叉反应-酸化处理后，可以检测Latent TGF- β 1复合物。干扰因素-重组小鼠TGF- β RII蛋白可以显著干扰该方法检测。

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
TGF- β 1 microplate	包被TGF- β 1抗体的96孔聚苯乙烯微孔板/8孔 $\times$ 12条	1瓶
TGF- β 1 Conjugate	酶标TGF- β 1检测抗体	1瓶
TGF- β 1 Standard	TGF- β 1标准品（冻干），参考瓶身标签进行重溶	2瓶
Calibrator Diluent（2 $\times$ ）/RD6-11	浓缩的标准品稀释液（2 $\times$ ）用于稀释标准品和样本	2瓶
Assay Diluent/RD1-21	检测液	1瓶
Wash Buffer Concentrate（25 $\times$ ）	浓缩洗涤液（25 $\times$ ）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	1瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

未开封试剂盒	2-8℃储存；请在试剂盒有效期内使用	
已打开，稀释或重溶的试剂	洗涤液（1×）	2-8℃储存，最多30天*。
	终止液	
	酶标检测抗体	
	检测液/RD1-21	
	TMB底物溶液	
	标准品	分装，-20℃储存最多30天*；避免反复冻融。
	标准品稀释液(2×)/RD6-11	2-8℃储存，最多 30 天* 请每次使用新鲜配制的 1×标准品稀释液，多余的丢弃
	包被的微孔板条	将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8℃储存，最多30天*。

*必须在试剂盒有效期内。

C. 实验所需自备试验器材

- 酶标仪（可测量450 nm检测波长的吸收值及540 nm或570 nm校正波长的吸收值）；
- 高精度加液器及一次性吸头；
- 蒸馏水或去离子水；
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机；
- 500 mL量筒；

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的保护。

VI. 实验前准备

A. 样品收集及储存

细胞培养上清液：颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于 $\leq -20^{\circ}\text{C}$ 冰箱内，避免反复冻融。样本可能需用稀释液（1×）稀释。

血清样本：用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后 $1000 \times g$ 离心15分钟。吸取血清样本之后即刻用于检测，或者分装， $\leq -20^{\circ}\text{C}$ 贮存备用。避免反复冻融。样本可能需用标准品稀释液（1×）稀释。

B. 样本活化

活化后的TGF- $\beta 1$ 在其免疫系统中可被检测，依据活化步骤如下表。检测样本需经过中和反应（pH 7.2-7.6），使用聚丙烯试管。

细胞培养上清液	血清
100 uL细胞培养上清液中加入20 ul的1N HCl	40 uL的血清中加入20 ul的1N HCl
混匀	混匀
室温孵育10 min	室温孵育10 min
加入20 uL的1.2 N NaOH/0.5 M HEPES中和酸化样本	加入20uL的1.2N NaOH/0.5 M HEPES中和酸化样本
混匀	混匀
立刻检测	检测样本前使用建议标准品稀释液（1×）稀释
读取样本，需要乘以稀释倍数1.4	样本检测需要乘以合适的稀释倍数*

*活化后的血清样本在使用前 $2-8^{\circ}\text{C}$ 可保存最多24小时，活化后的细胞培养上清样本必须立刻检测。不可冷冻活化样本。

C. 样本准备工作

细胞上清样本建议用活化后1.4倍稀释后进行检测，活化后的细胞培养基样本即为1.4倍立刻检测。最佳稀释度应由最终用户确定。

*血清样本活化后建议用标准品稀释液（1×）至少60倍稀释后进行检测，例如：10 μL 活化血清样本+90 μL标准品稀释液（1×）中，充分混匀，即10倍稀释。然后取20 μL 10倍稀释后的样本加到100 μL标准品稀释液（1×）中，充分混匀，即制备成60倍稀释的样本。（最终的稀释倍数是120倍。）最佳稀释度应由最终用户确定。

D. 检测前准备工作

使用前请将所有试剂放置于室温。

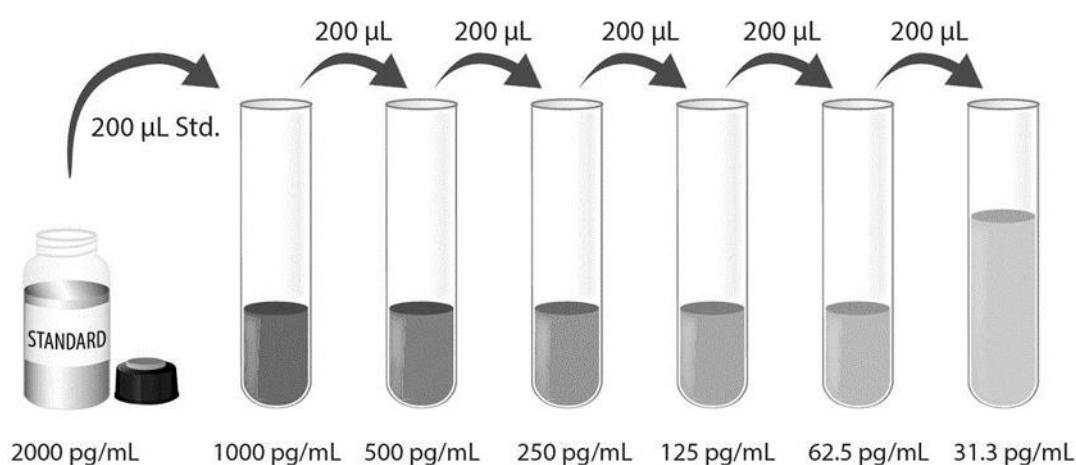
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液（1×）：使用去离子水或蒸馏水稀释配制成标准品稀释液（1×）。

TGF-1标准品：参照标准品瓶身注明的方式重溶冻干标准品。用标准品稀释液（1×）重溶冻干标准品，得到浓度为2000 pg/mL标准品母液。轻轻震荡至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

使用聚丙烯试管作为标准品稀释管。每管加入200 μL标准品稀释液（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。没有稀释的标准品母液可用作标准曲线最高点（2000 pg/mL），标准品稀释液（1×）可用作标准曲线零点（0 pg/mL）。



E. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 为了获得好结果，将试剂盒样品上样到每孔中间；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- 当使用自动洗板机时，加入洗涤液后浸没30秒，或者每步清洗之间水平旋转180度，这可以提高其测定精度；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀请充分混合。

VII. 操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 在每个微孔中加入50 μL 检测液；
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔50 μL 。用封板膜封住反应孔，**室温孵育2小时**。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 μL ，然后将板内洗涤液吸去。重复操作3次，共洗4次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
6. 在每个微孔内加入100 μL 酶标检测抗体。用封板膜封住反应孔，**室温孵育2小时**；
7. 重复第5步洗板操作；
8. 在每个微孔内加入100 μL TMB底物溶液，**室温孵育30分钟。注意避光**；
9. 在每个微孔内加入100 μL 终止液，请轻拍微孔板，使溶液混合均匀；
10. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确；
11. **计算结果：**将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制TGF- β 1浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

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96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置

