PRODUCT INFORMATION & MANUAL

Mouse/Rat/Porcine/Canine TGF-β1 Valukine™ ELISA

VAL611

For the quantitative determination of natural and recombinant Mouse/Rat/Porcine/Canine TGF-β1 concentrations

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

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Novus kits are guaranteed for 3 months from date of receipt

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I. BACKGROUND

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).

II. OVERVIEW

A. 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 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 monoclonal antibody specific for TGF-β1 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-β1 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 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 Diluent 1× 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
n	20	20	20	20	20
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-β1 spiked to different levels throughout the range of the assay was evaluated. The recovery ranged from 83-114% with an average of 105%.

The recovery of TGF-β1 spiked to different levels throughout the range of the assay in serum was evaluated. The recovery ranged from 74.5-90.2% with an average of 78.9%.

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse TGF-β1 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-β1 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-β1 and diluted with Diluent 1× 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-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 (1×10^6 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 (1×10^6 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 (1×10^6 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.

Serum - Four Serum samples were evaluated for the presence of TNF-β1 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-β1. This assay also recognizes human TGF-β1.

The factors listed below were prepared at 50 ng/mL in Diluent 1x 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
Activin RIA
Activin RIIA
Activin RIIB
BMP-2
BMP-3
BMP-3b
BMP-4
BMP-5
BMP-6
BMP-8b
BMP-10
BMP-15
BMPR-IA
BMPR-IB
BMPR-II
Follistatin288
Follistatin300
Follistatin315
Inhibin A
Inhibin B
LAP (TGF-β1)
TGF-α
TGF-β RI
TGF-β RII
TGF-β RIII
TGF-β2
TGF-β3

Recombinant Mouse

BMP-3b
TGF-β RI
BMPR-IA
BMPR-IB

Other Recombinant

Rat Agrin
Zebrafish BMP-2a
Amphibian TGF-β5

Nature protein

Porcine TGF-β2

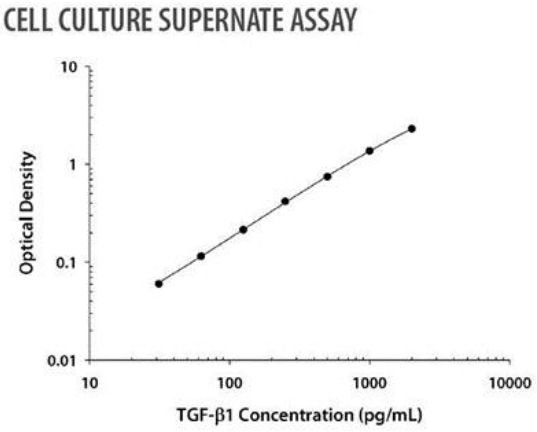
Cross-reactivity - This kit detects Latent TGF-β1 complex after acid treatment.

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

IV. EXPERIMENT

EXAMPLE STANDARD

The 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	0.050 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

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
TGF-β1 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against TGF-β1	1 plate
TGF-β1 Conjugate	Solution of polyclonal antibody against TGF-β1 conjugated to horseradish peroxidase with preservatives	1 vial
TGF-β1 Standard	Recombinant TGF-β1 in a buffered protein base with preservatives; lyophilized; Refer to the vial label for reconstitution volume	2 vial
Calibrator Diluent (4×)	A 4× concentrated buffered protein base with preservatives	1 vial
Assay Diluent	A buffered protein base with preservatives	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives	1 vial
Color Reagent A	Stabilized hydrogen peroxide	1 vial
Color Reagent B	Stabilized chromogen (tetramethylbenzidine)	1 vial
Stop Solution	Diluted hydrochloric acid	1 vial
Plate Covers	Adhesive strips	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8 °C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/ Reconstituted Reagents</td><td>Diluted Wash Solution</td><td rowspan="7">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Diluent 1×</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Assay Diluents</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the oil ouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*</td></tr>
</table>

* 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

- 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.

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 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C C. Avoid repeated freeze-thaw cycles.

B. SAMPLE ACTIVATION PROCEDURE

To activate latent TGF-β1 to immunoreactive TGF-β1 detectable by the TGF-β1 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 µL of cell culture supernate, add 20 µL of 1 N HCl.	To 40 µL serum, add 20 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 20 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 Reagent Diluent 1(diluted). 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 require at least a 1.4-fold dilution prior to the assay. A suggested 1.4-fold dilution is activation of the sample.

*Activated Serum samples require 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.).

D. REAGENT PREPARATION

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

Wash Solution - 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.

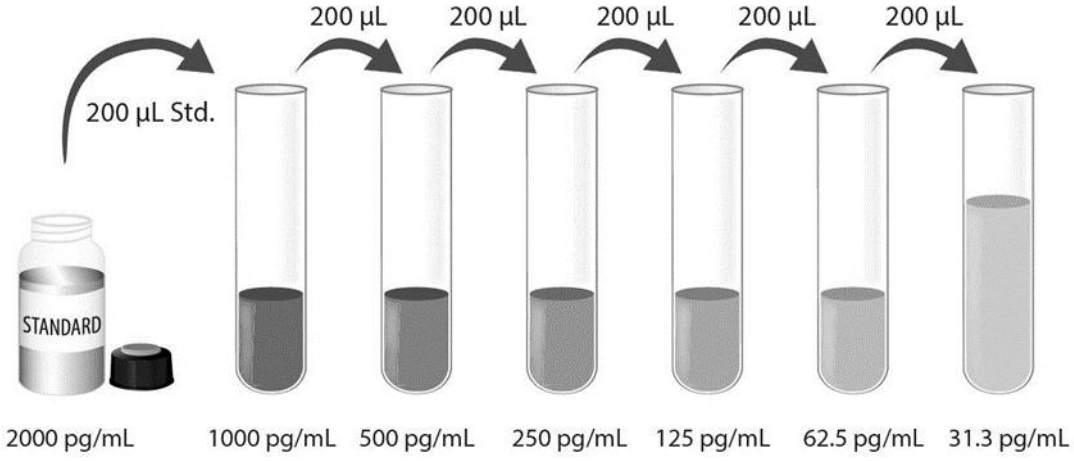
Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Diluent 1× - Add 20 mL of Calibrator Diluent 4× into 60 mL of deionized or distilled water to prepare 80 mL of Diluent 1×.

Mouse TGF-1 Standard - Refer to the vial label for reconstitution volume*. Reconstitute the TGF-β1 Standard with 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 Diluent 1x 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). Diluent 1x 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.
- 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.

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 µL of Assay Diluent to each well.
4. Add 50 µL of Standard, sample, or control 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 Solution (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 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. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, 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.
11. **CALCULATION OF RESULTS.** Average the duplicate readings for each standard, control, 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-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.

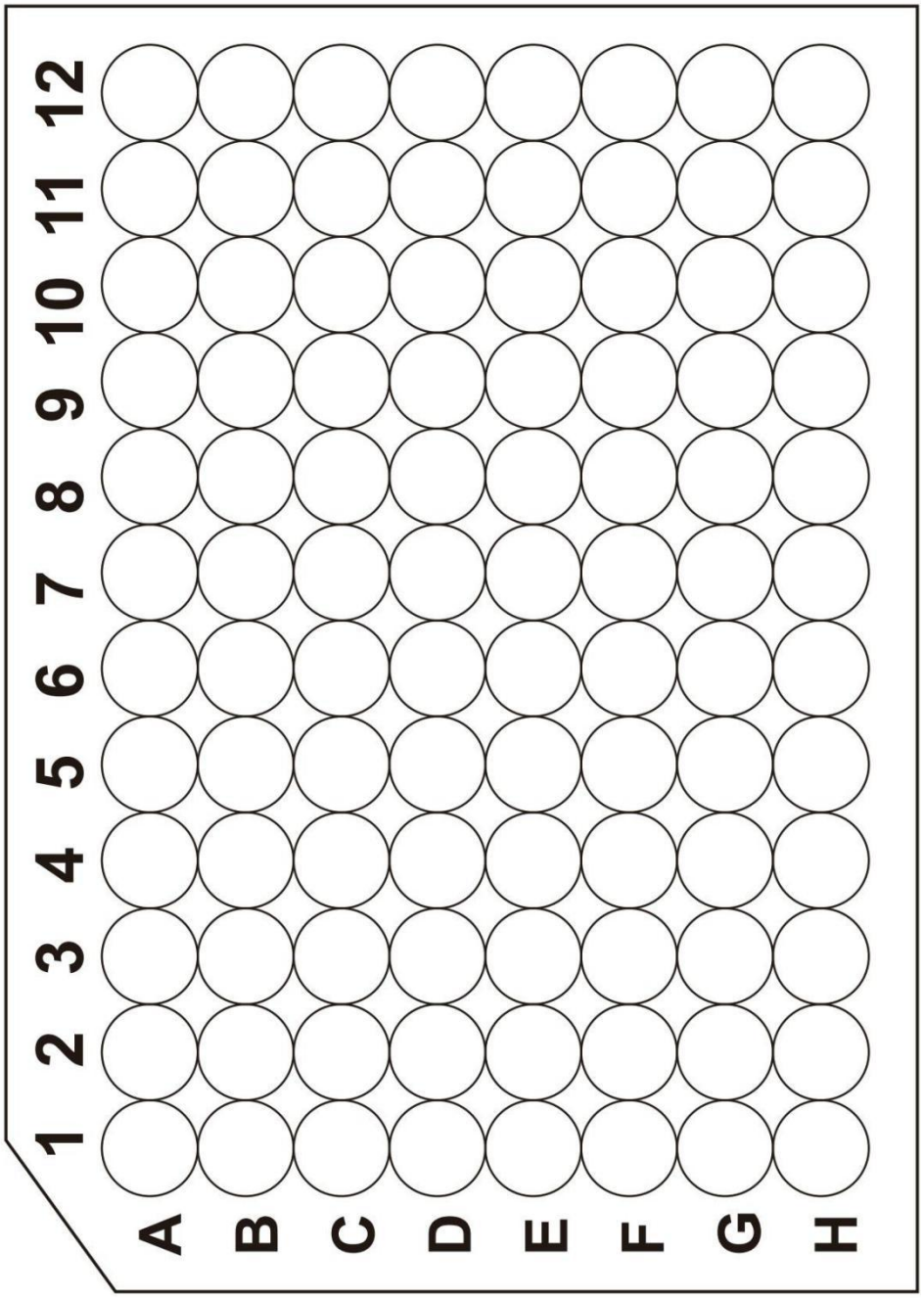
    If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. (For example, if the Cell culture supernate samples require to be activated (1.4 times) for detection, the concentration must be multiplied by the activation coefficient 1.4. Activated serum samples (2 times) requires 60 times dilution with reagent dilution (1×), and the concentration must be multiplied by the activation and dilution factor of 120.).

VIII. RERERENCES

1. Kingsley, D.M. (1994) Genes Dev. 8:133.
2. Javelaud, D. and A. Mauviel (2004) Int. J. Biochem. Cell Biol. 36:1161.
3. Chang, H. et al. (2002) Endocr. Rev. 23:787.
4. Kondaiah, P. et al. (1988) J. Biol. Chem. 263:18313.
5. Clark, D.A. and R. Coker (1998) Int. J. Biochem. Cell Biol. 30:293.
6. Qian, S.W. et al. (1990) Nucleic Acids Res. 18:3059.
7. Derynack, R. et al. (1986) J. Biol. Chem. 261:4377.
8. Dubois, C.M. et al. (1995) J. Biol. Chem. 270:10618.
9. Manning, A.M. et al. (1995) Gene 155:307.
10. Brunner, A.M. et al. (1989) J. Biol. Chem. 264:13660.
11. Mittl, P.R.E. et al. (1996) Protein Sci. 5:1261.
12. Gray, A.M. and A.J. Mason (1990) Science 247:1328.
13. Oklu, R. and R. Hesketh (2000) Biochem. J. 352:601.
14. Saharinen, J. et al. (1996) EMBO J. 15:245.
15. Mangasser-Stephan, K. and A.M. Gressner (1999) Cell Tissue Res. 297:363.
16. Koli, K. et al. (2001) J. Cell Sci. 114:2869.
17. Yang, L. et al. (2000) Wound Rep. Reg. 8:538.
18. Pedrozo, H.A. et al. (1999) Endocrinology 140:5806.
19. Yu, Q. and I. Stamenkovic (2000) Genes Dev. 14:163.
20. Maeda, S. et al. (2001) J. Bone Miner. Res. 16:1281.
21. Munger, J.S. et al. (1999) Cell 96:319.
22. Derynck, R. et al. (1985) Nature 316:701.
23. Derynck, R. and X-H. Feng (1997) Biochim. Biophys. Acta 1333:F105.
24. Ten Dijke, P. et al. (1996) Curr. Opin. Cell. Biol. 8:139.
25. Chai, Y. et al. (2003) Crit. Rev. Oral Biol. Med. 14:78.
26. De Caestecker, M. et al. (2004) Cytokine Growth Factor Rev. 15:1.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠/大鼠/猪/犬TGF-β1 Valukine™ ELISA 试剂盒

目录号：VAL611

适用于定量检测天然和重组小鼠/大鼠/猪/犬TGF-β1的含量

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目录

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-β1，-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，后者其中还包括激活素/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βTGF-β1 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 个氨基酸的潜在期的相关肽（LAP），和一个 C 末端 112 个氨基酸的成熟 TGF-β1（4-9）。LAP 和成熟的TGF-β1 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潜在型TGF-β1 复合物。在大多数细胞类型，这种复合物也可通过 LAP 共价结合一个潜在的TGF-β1 结合蛋白（LTBP）。这会产生一个分泌型的、巨大的潜在复合物。存在于小的或大的潜在复合物中的 TGF-β1，不能用于TGF-β受体的结合和激活，因此是潜在的。而LAP 对于转化潜在型到TGF-β1 是必要的和足够的，LTBP 促进了小的潜在复合物的正确折叠和分泌。LTBP 也是细胞外基质的结构成分，它引导了潜在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异构体的小的潜在复合体（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-β1 显示与成熟大鼠TGF-β1 的100%氨基酸序列同源性（6, 7）。成熟大鼠和小鼠的TGF-β1 与成熟猪、人和犬的TGF-β1 显示99% 氨基酸序列同源性（4, 9, 22）。成熟猪TGF-β1 与成熟的人和犬TGF-β1 具有绝对的同源性。

TGF-β1 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-βRI）和II 型（TGF-βRII）跨膜丝氨酸/苏氨酸激酶受体。TGF-βRII 是结构性活化激酶。一旦结合 TGF-β1，它便磷酸化和激活TGF-βRI。接着，TGF-βRI 磷酸化和激活了调节转录的Smad 蛋白。

TGF-βRI，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-βRII 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-βRIII（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-β1 单抗包被于微孔板上，样品和标准品中的 TGF-β1 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抗人 TGF-β1 多抗，未结合的抗体被洗去；加入底物溶液（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，应将样本用稀释剂（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、试剂盒的储存等。

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

在细胞培养基活化样本中掺入检测范围内不同水平的TGF-β1，测定其回收率。回收率范围在83-114%， 平均回收率在 105%。

在小鼠血清样本中掺入检测范围内不同水平的TGF-β1，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-β1 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 表达的高纯度重组人TGF-β1 蛋白所校正。

E. 线性

不同的活化样本中掺入高浓度的TGF-β1，然后用稀释剂（1×）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-β1 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-β1 量。

按 1×10^6 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 μg/mL PHA 和 10 ng/mL PMA，培养 3 天后，取上清检测TGF-β1。

按 1×10^6 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 100 ng/mL LPS，培养 24h 后，取上清检测TGF-β1。

按 1×10^6 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-β1。

血清样本 - 使用本试剂盒检测了4份小鼠血清样本中TNF-β1的水平。4份样本的检测值在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-β1 蛋白，也可识别人TGF-β1 和TGF-β2。将以下因子用稀释剂（1×）配置成 50 ng/mL 的浓度来检测交叉反应。将 50ng/mL 的干扰因子掺入中间范围的TGF-β1 对照品中，来检测干扰。没有观察到明显的交叉反应或干扰。

重组人蛋白

Activin A
Activin RIA
Activin RIIA
Activin RIIB
BMP-2
BMP-3
BMP-3b
BMP-4
BMP-5
BMP-6
BMP-8b
BMP-10
BMP-15
BMPR-IA
BMPR-IB
BMPR-II
Follistatin288
Follistatin300
Follistatin315
Inhibin A
Inhibin B
LAP (TGF-β1)
TGF-α
TGF-β RI
TGF-β RII
TGF-β RIII
TGF-β2
TGF-β3

重组小鼠蛋白

BMP-3b
TGF-β RI
BMPR-IA
BMPR-IB

其他重组蛋白

大鼠 Agrin
斑马鱼 BMP-2a
两栖动物 TGF-β5

天然蛋白

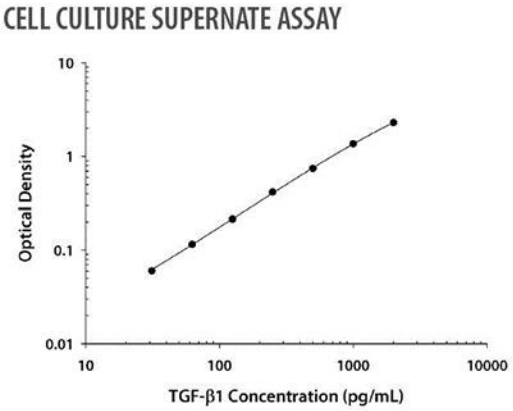
猪TGF-β2

交叉反应-酸化处理后，可以检测惰性 TGF-β1 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

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。



(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

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
TGF-β1 microplate	包被TGF-β1抗体的 96 孔聚苯烯微孔板/ 8 孔×12 条	1 瓶
TGF-β1 Conjugate	酶标检测抗体	1 瓶
TGF-β1 Standard	标准品（冻干）	2 瓶
Calibrator Diluent（4×）	浓缩稀释剂（4×）	1 瓶
Assay Diluent	检测液	1 瓶
Wash Buffer Concentrate（25×）	浓缩洗涤液（25×）	1 瓶
Color Reagent A	显色剂 A	1 瓶
Color Reagent B	显色剂 B	1 瓶
Stop Solution2	终止液	1 瓶
Plate Covers	封板胶纸	3 张

* 本试剂盒包含足够的试剂以用于一块 96 孔微孔板的ELISA 实验。

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="9">已打开，稀释或重溶的试剂</td><td>洗涤缓冲液（1×）</td><td rowspan="7">2-8℃储存，最多 30 天*。</td></tr>
<tr><td>终止液</td></tr>
<tr><td>稀释剂（1×)</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>检测液</td></tr>
<tr><td>显色剂 A</td></tr>
<tr><td>显色剂 B</td></tr>
<tr><td>标准品</td><td>分装，-20℃手动除霜储存最多 30 天*；避免反复冻融。</td></tr>
<tr><td>包被的微孔板条</td><td>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*。</td></tr>
</table>

*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；
- 100 mL 和500 mL 量筒；

D. 注意事项

- 试剂盒中的终止液是酸性溶液，使用时请做好眼镜、手、面部及衣服的防护。
- Color Reagent B 可能会刺激皮肤、眼睛和呼吸道。请避免吸入挥发气体。

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-20℃冰箱内，避免反复冻融。样本可能需要用稀释剂（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1000x g离心15分钟。吸取血清样本之后即刻用于检测，或者分装，-20℃贮存备用。避免反复冻融。

B. 样本活化

活化后的TGF-β1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。

细胞培养基	**血清**
100 uL培养基中加入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10min	室温孵育10min
加入20uL的1.2N 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试剂稀释剂（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℃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

细胞上清样本需要用活化后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。

*血清样本活化后需要用稀释剂（1×）至少60倍稀释后进行检测，例如：10μL活化血清样本+90μL稀释剂（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稀释剂（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。）

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：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 浓缩洗涤液用蒸馏水或去离子水稀释配置成500 mL 工作浓度的洗涤液。未用完的放回 4℃。

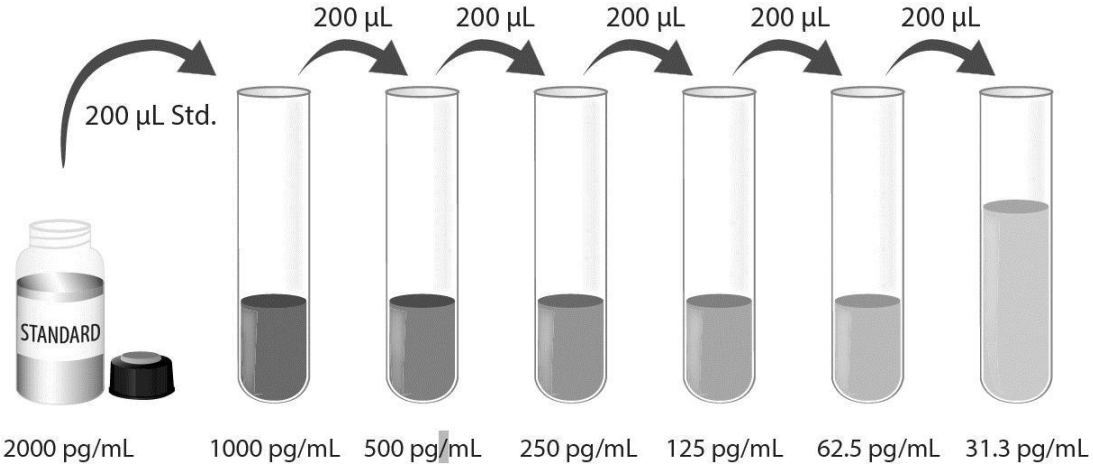
显色剂：按当次试验所需要用量将显色剂 A 和显色剂B 等体积混合，避光；在使用前15 分钟内准备，仅供当日使用；每孔需 100 μL。

稀释剂（1×）：可将 20mL 浓缩稀释剂（4×）用 60mL 蒸馏水或去离子水稀释配置成 80 mL 工作浓度的稀释剂。

标准品：参照标准品瓶身注明的方式重溶冻干标准品。用稀释剂（1×) 重溶冻干标准品， 得到浓度为 2000 pg/mL 标准品母液。轻轻震摇至少 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 稀释剂（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），稀释剂（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，配置不同浓度标准品、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哦每孔中间；
- 建议 15 分钟内完成一块板的上样；
- 每次孵育时，正确使用封板胶纸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；
- 混合后的显色底物在上板前应为无色，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显色底物上板顺序一致；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，实验样本或者质控品加入相应孔中，每孔 50 µL。用封板胶纸 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 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。用封板胶纸封住反应孔，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 显色底物，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，孔内溶液颜色会从蓝色变为黄色。如果溶液颜色变为绿色或者颜色变化不一致，请轻拍微孔板，使溶液混合均匀；
10. 加入终止液后 30 分钟内，使用酶标仪测量 450 nm 的吸光度值，设定 540 nm 或570nm 作为校正波长。如果没有使用双波长校正，结果准确度可能会受影响；
11. **计算结果**：将每个标准品和样品的校正吸光度值(OD450-OD540/OD570)、复孔读数取平均值，然后减去平均零标准品 OD 值。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。另一种方法是，可以通过绘制标准品浓度做对数与相应 OD 值对数生成曲线，并通过回归分析确定最佳拟合线。这个过程可生成一个足够使用但不太精确的数据拟合。若样本经过稀释，计算浓度时应乘以稀释倍数。（如：细胞上清样本需要活化（1.4倍）后进行检测，则浓度必须乘以活化系数1.4；血清样本活化（2倍）后需要用试剂稀释剂 1（1×）60倍稀释后进行检测，则浓度必须乘以活化和稀释系数120。）

VIII. 参考文献

1. Kingsley, D.M. (1994) Genes Dev. 8:133.
2. Javelaud, D. and A. Mauviel (2004) Int. J. Biochem. Cell Biol. 36:1161.
3. Chang, H. et al. (2002) Endocr. Rev. 23:787.
4. Kondaiah, P. et al. (1988) J. Biol. Chem. 263:18313.
5. Clark, D.A. and R. Coker (1998) Int. J. Biochem. Cell Biol. 30:293.
6. Qian, S.W. et al. (1990) Nucleic Acids Res. 18:3059.
7. Derynack, R. et al. (1986) J. Biol. Chem. 261:4377.
8. Dubois, C.M. et al. (1995) J. Biol. Chem. 270:10618.
9. Manning, A.M. et al. (1995) Gene 155:307.
10. Brunner, A.M. et al. (1989) J. Biol. Chem. 264:13660.
11. Mittl, P.R.E. et al. (1996) Protein Sci. 5:1261.
12. Gray, A.M. and A.J. Mason (1990) Science 247:1328.
13. Oklu, R. and R. Hesketh (2000) Biochem. J. 352:601.
14. Saharinen, J. et al. (1996) EMBO J. 15:245.
15. Mangasser-Stephan, K. and A.M. Gressner (1999) Cell Tissue Res. 297:363.
16. Koli, K. et al. (2001) J. Cell Sci. 114:2869.
17. Yang, L. et al. (2000) Wound Rep. Reg. 8:538.
18. Pedrozo, H.A. et al. (1999) Endocrinology 140:5806.
19. Yu, Q. and I. Stamenkovic (2000) Genes Dev. 14:163.
20. Maeda, S. et al. (2001) J. Bone Miner. Res. 16:1281.
21. Munger, J.S. et al. (1999) Cell 96:319.
22. Derynck, R. et al. (1985) Nature 316:701.
23. Derynck, R. and X-H. Feng (1997) Biochim. Biophys. Acta 1333:F105.
24. Ten Dijke, P. et al. (1996) Curr. Opin. Cell. Biol. 8:139.
25. Chai, Y. et al. (2003) Crit. Rev. Oral Biol. Med. 14:78.
26. De Caestecker, M. et al. (2004) Cytokine Growth Factor Rev. 15:1.

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