PRODUCT INFORMATION & MANUAL

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

Catalog Number: VAL611

For the quantitative determination of natural and recombinant Human/Mouse/Rat/Porcine/Canine TGF-β1 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 202604.10

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I. BACKGROUND

Human TGF-β1 is a 25 kDa, disulfide-linked homodimeric protein involved in a number of key developmental, immunologic, and homeostatic processes (1-4). The molecule is synthesized as a 390 amino acid (aa) precursor that contains a 23 aa signal sequence, a 255 aa pro-region, and a 112 aa mature segment. Processing of the molecule is complex, and it is generally secreted as a latent form (5). Prior to release, the prepro-form is cleaved of its signal sequence, followed by glycosylation of its proregion. The glycosylation process includes the unusual attachment of mannose-6 phosphate residues. This is followed by furin convertase-mediated cleavage of the prohormone, creating an 80 kDa disulfide-linked proregion (termed LAP for latency-associated protein), plus a 25 kDa disulfidelinked mature segment (termed TGF-β1) (6-8). These two independent disulfide-linked polypeptides associate in a non-covalent interaction that renders TGF-β1 inactive. Although direct secretion of this 80K:25K complex can occur, it does so inefficiently. To facilitate secretion plus extracellular storage, a third 200 kDa component termed LTBP is covalently-linked to the N-terminus of one of the two LAP polypeptide chains. This promotes secretion and subsequent storage within the extracellular matrix (9, 10). After secretion, TGF-β1, via LTBP, covalently links to ECM. This complex is later cleaved by proteases and released, exposing mannose residues on LAP. It is postulated that exposed LAP mannose residues now are able to bind to cell surface IGF-II R, where dissociative events disrupt the LAP-TGF-β1 complex. This results in the release of active, homodimeric TGF-β1 (7, 10). Mature mouse TGF-β1 shares 100% aa sequence identity with rat and cotton rat TGF-β1 (11, 12), 99% aa identity with human, canine, and porcine TGF-β1 (13, 14, 15), and 97% aa identity with guinea pig TGF-β1 (16). Relative to mouse TGF-β2 and β3, mature mouse TGF-β1 shares 72% and 78% aa sequence identity, respectively (17, 18).

The traditional high-affinity receptor for TGF-β1 is a heteromeric complex consisting of transmembrane serine/threonine kinases. Two types are involved; a constitutively phosphorylated, ligand-binding 80 kDa glycoprotein termed TGF-β1 RII and a signal-transducing, non-ligand-binding 55 kDa glycoprotein termed TGF-β RI/ALK-5 (19-22). It is suggested that TGF-β1 first binds TGF-β1 RII, which then initiates a cross-phosphorylation of TGF-β1 RI, culminating in signal transduction. There is also a third TGF-β receptor termed TGF-β1 RIII, which can be either the 250 kDa

proteoglycan named betaglycan, or the 180 kDa glycoprotein termed endoglin/CD105 (23, 24). It has been proposed that TGF-β1 RIII captures TGF-β and "passes" it to TGF-β1 RII (20). This is perhaps true for betaglycan but not endoglin. Endoglin does not bind TGF-β by itself; only within the context of TGF-β1 RII ligand binding. Evidence suggests that rather than "passing" on ligand, endoglin may actually enter the receptor complex and modulate TGF-β downstream signaling (25, 26). Finally, and although ALK-5 has traditionally been assumed to be the only type I signaling receptor for TGF-β1, it is also possible that ALK-1 may serve as a condition-dependent, type I TGF-β receptor (27).

TGF-β1 has a wide range of activities. During an immune response, TGF-β1 impacts antibody production by preferentially inducing IgA production in both mouse and human (28). It also regulates dendritic cell chemotaxis by altering the expression of chemokine receptors (29). Finally, it can downmodulate an inflammatory response by dampening macrophage activity and proinflammatory cytokine secretion (30). During wound healing, TGF-β1 is released from activated platelets. This local source of TGF-β1 has marked stimulatory effects on fibroblasts, where it induces matrix synthesis; on monocytes, where it induces proinflammatory mediator and growth factor secretion; and on keratinocytes, where it may promote keratinocyte proliferation by downmodulating its own signaling pathway (31). Finally, during development, TGF-β1 may play a role in endochondral ossification, and its absence results in severely defective yolk sac vasculogenesis and hematopoiesis (32, 33).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An 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 antibody specific for TGF-β1 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-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 human/mouse/rat/porcine/canine serum, human urine, human/mouse/rat/porcine/canine platelet-poor plasma.
- 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×) 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)

Three 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.

CELL CULTURE SUPERNATE ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	317	683	1271	312	657	1184
Standard Deviation	11.3	42.2	31.1	26.1	46.6	92.3
CV%	3.6	6.2	2.4	8.4	7.4	7.8

SERUM/PLASMA ASSAY

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

B. RECOVERY

The recovery of TGF-β1 spiked to levels throughout the range of the assay in activated sampleswas evaluated.

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

C. SENSITIVITY

Thirty-three assays were evaluated and the minimum detectable dose (MDD) of human/mouse/rat/porcine/canine TGF-β1 ranged from 0.889-5.50 pg/mL. The mean MDD was 2.38 pg/mL.

The MDD was determined by adding two standard deviations to the mean 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-β1produced at R&D Systems®.

The NIBSC/WHO TGF-β1 International Standard 89/514 (Human, rDNA Derived) was evaluated in this kit. The dose response curve of the reference reagent 89/514 parallels the Valukine standard curve. To convert sample values obtained with the Valukine Human/Mouse/Rat/Porcine/Canine TGF-β1 kit to approximate NIBSC/WHO 89/514 Units, use the equation below:

Cell culture supernate/Urine - NIBSC/WHO 89/514 approximate value (IU/mL) = 0.0159 × Valukine Human/Mouse/Rat/Porcine/Canine TGF-β1 value (pg/mL)

Serum/Platelet-poor Plasma - NIBSC/WHO 89/514 approximate value (IU/mL) = 0.0171 × Valukine Human/Mouse/Rat/Porcine/Canine TGF-β1 value (pg/mL)

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

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of TGF-β1 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

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

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

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

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

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

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

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

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

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

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

F. SAMPLE VALUES

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

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

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

ND=Non-detectable

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

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

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

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

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

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

Cell Culture Supernates - Human peripheral blood mononuclear cells (PBMCs) were separated from whole blood by a density gradient centrifugation method using Ficoll-Paque Plus. $CD4^+$ T cells were isolated from PBMCs using the MagCellect™ Human $CD4^+$ T cell Isolation Kit (R&D Systems®, Catalog # MAGH102). Cells were seeded at 5 x 10^5 /mL and cultured using Excellerate™ Human T Cell Expansion Media, Xeno-Free (R&D Systems, Catalog # CCM030). T cells were left untreated or treated with 10 ng/mL GMP recombinant human (rh) IL-7 (R&D Systems, Catalog # 207-GMP), 10 ng/mL GMP rhIL-15 (R&D Systems, Catalog # 247-GMP), and stimulated via their T cell receptor (TCR) and co-stimulatory receptor for 5 days. TCR stimulation was mediated using 25 µL Cloudz™ CD3/28 particles (Cloudz T Cell Activation Kit - CD3/CD28, (R&D Systems, Catalog # CLD001) per mL of culture

media. $CD4^+$ T cells were maintained in a 5% CO_2 incubator at 37 °C for 5 days. An aliquot of the cell culture supernates was removed, assayed for TGF-β1, and measured 77.7 pg/mL (untreated) and 471 pg/mL (treated).

Human PBMCs were seeded at 1 x 10^6 /mL and cultured in RPMI supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin and left untreated. An aliquot of the cell culture supernate was removed, assayed for TGF-β1, and measured 2854 pg/mL.

Mouse EL-4 cells were cultured in DMEM High Glucose supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were then treated with 10 ng/mL PMA and 10 ug/mL PHA for 24 hours. An aliquot of the cell culture supernate was removed, assayed for TGF-β1, and measured 2969 pg/mL.

Rat spleen was taken from a pregnant Sprague Dawley rat, homogenized, and cultured in DME with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C and 5% CO_2. Rat splenocytes were treated with 50 ng/mL recombinant rat IL-2 and 5 µg/mL PHA for 3 days. An aliquot of the cell culture supernate was removed, assayed for TGF-β1, and measured 2716 pg/mL.

Porcine PK-15 cells were cultured in MEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were treated with 1 µg/mL LPS for 72 hours. An aliquot of the cell culture supernate was removed, assayed for TGF-β1, and measured 1631 pg/mL.

G. 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 (1×) 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		**Recombinant mouse**	**Other recombinant**
Activin A	BMPR-IB	Activin RI	rat Agrin
Activin RIA	BMPR-II	Activin RIIA	zebrafish BMP-2
Activin RIIA	$Follistatin_{288}$	Activin RIB	amphibian TGF-β5
BMPR-IB	$Follistatin_{300}$	Activin RIIB	chinese hamster TGF-β1-LAP
Activin RIIB	$Follistatin_{315}$	BMP-3	
Agrin	GDF-5	BMP-3b	**Natural protein**
BMP-1.1	GDF-7	BMP-4	porcine TGF-β2
BMP-2	GDF-9	BMP-6	
BMP-3	GDF-11	BMP-7	
BMP-3b	GDF-15	BMP-9	
BMP-4	Inhibin A	BMPR-IA	
BMP-5	Inhibin B	BMPR-IB	
BMP-6	LAP (TGF-β1)	BMPR-II	
BMP-7	TGF-α	$Follistatin_{288}$	
BMP-8a	TGF-β RI	Inhibin A	
BMP-8b	TGF-β RIII	TGF-β RI	
BMP-10	TGF-β3	TGF-β RIII	
BMP-15			
BMPR-IA			

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

Recombinant Protein	Assay Diluent	
	RD1-21	RD1-73
Human BMP-9	<1%	<1%
Mouse BMP-10	<1%	<1%
Human Latent TGF-β1	<1%	<1%
Activated Human Latent TGF-β1	15%	15%
Equine TGF-β1	3%	<1%
Human TGF-β1.2	14%	12%
Human TGF-β2	13%	12%

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

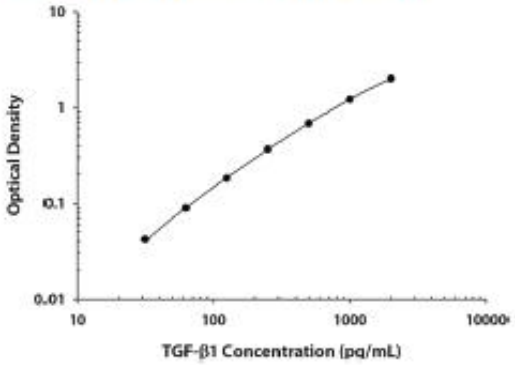
Human or Mouse TGF-β RII (ng/mL)	TGF-β1 (pg/mL)	% TGF-β1 Detected in presence of Human TGF-β RII		% TGF-β1 Detected in presence of Mouse TGF-β RII	
		RD1-21	RD1-73	RD1-21	RD1-73
0	500	100	100	100	100
1.56	500	98	99	90	93
3.13	500	93	95	85	88
6.25	500	82	86	77	79
12.5	500	63	66	69	67
25	500	42	37	60	55
50	500	28	18	52	40

IV. EXPERIMENT

EXAMPLE STANDARD

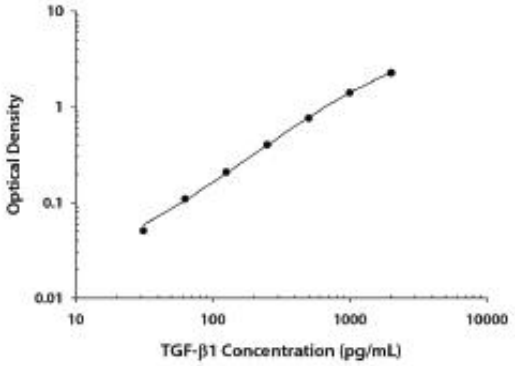
The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/URINE ASSAY



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

SERUM/PLATELET-POOR PLASMA ASSAY



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

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 an antibody against TGF-β1	1 plate
TGF-β1 Conjugate	Solution of 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 vials
Calibrator Diluent (2×) RD6-11	A 2× concentrated buffered protein base used to dilute standard and samples	1 vial
Assay Diluent RD1-21	A buffered protein base with preservatives. *For cell culture supernate/urine samples*	1 vial
Assay Diluent RD1-73	A buffered base with preservatives. *For serum/platelet-poor plasma samples*	1 vial
Wash Buffer Concentrate (25×)	A 25× 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

<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="8">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="6">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Assay Diluent RD1-21</td></tr>
<tr><td>Assay Diluent RD1-73</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>Use a new standard for each assay. Discard after use.</td></tr>
<tr><td>Calibrator Diluent (2×) RD6-11</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>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.*</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

- 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

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.*

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

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

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

Note: *Neat unactivated urine samples exhibit a decrease in TGF-β1 concentration in the first 24 hours of storage (frozen or refrigerated). Care should be taken that samples are assayed under identical storage conditions and durations.*

Platelet-poor Plasma* - Collect plasma on ice using EDTA or heparin as an

anticoagulant. Centrifuge for 20 minutes at 1000 × g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay (see activation procedure) immediately or aliquot and store samples at ⩽ -20 °C. Avoid repeated freeze-thaw cycles.

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

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

B. SAMPLE ACTIVATION PROCEDURE

ACTIVATION REAGENT PREPARATION

To activate latent TGF-β1 to immunoreactive form, prepare the following solutions for acid activation and neutralization. The solutions may be stored in polypropylene bottles at room temperature for up to one month.

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

1 N HCl (100 mL) - To 91.67 mL of deionized water, slowly add 8.33 mL of 12 N HCl. Mix well.

1.2 N NaOH/0.5 M HEPES (100 mL) - To 75 mL of deionized water, slowly add 12 mL of 10 N NaOH. Mix well. Add 11.9 g of HEPES. Mix well. Bring final volume to 100 mL with deionized water.

For each new lot of acidification and neutralization reagents, measure the pH of several representative samples after neutralization to ensure that it is within pH 7.2-7.6. Adjust the volume and corresponding dilution factor of the neutralization

reagent as needed.

TGF-β1 SAMPLE ACTIVATION PROCEDURE

To activate latent TGF-β1 to immunoreactive TGF-β1 detectable by the TGF-β1 immunoassay, follow the activation procedure below. Assay samples is pH 7.2-7.6 after neutralization. **Use polypropylene test tubes.**

Note: ***Do not activate the kit standards.*** *The standards contain active recombinant TGF-β1.*

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.
Prior to the assay, dilute the activated sample with calibrator diluent (1×). *See the following for suggested dilutions.*	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.

C. SAMPLE PREPARATION

Cell culture supernate samples (human and non-human) tested neat, but may require dilution if high endogenous levels are present (final dilution factor of sample is 1.4 if tested neat). Optimal dilutions should be determined by the end user.

Human serum samples require a 40-fold dilution in Calibrator Diluent (1×)[†]. A suggested 40-fold dilution is 10 µL of activated sample + 390 µL of Calibrator Diluent

(1×) (final dilution factor of sample is 60). Optimal dilutions should be determined by the end user.

Human urine samples tested neat (final dilution factor of sample is 1.4). Optimal dilutions should be determined by the end user.

Human platelet-poor plasma* samples require a 8-fold dilution in Calibrator Diluent (1×)[†]. A suggested 8-fold dilution is 25 µL of activated sample + 175 µL of Calibrator Diluent (1×) (final dilution factor of sample is 12). Optimal dilutions should be determined by the end user.

Mouse and rat serum/platelet-poor plasma* samples require a 60-fold dilution in Calibrator Diluent (1×)[†]. A suggested 60-fold dilution is 10 µL of activated sample + 590 µL of Calibrator Diluent (1×) (final dilution factor of sample is 90). Optimal dilutions should be determined by the end user.

Porcine serum samples require a 15-fold dilution in Calibrator Diluent (1×). A suggested 15-fold dilution is 10 µL of activated sample + 140 µL of Calibrator Diluent (1×) (final dilution factor of sample is 22.5). Optimal dilutions should be determined by the end user.

Porcine platelet-poor plasma* samples require a 5-fold dilution in Calibrator Diluent (1×). A suggested 5-fold dilution is 40 µL of activated sample + 160 µL of Calibrator Diluent (1×) (final dilution factor of sample is 7.5). Optimal dilutions should be determined by the end user.

Canine serum and platelet-poor plasma* samples require a 40-fold dilution in Calibrator Diluent (1×). A suggested 40-fold dilution is 10 µL of activated sample + 390 µL of Calibrator Diluent (1×) (final dilution factor of sample is 60). Optimal dilutions should be determined by the end user.

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

[†] See Reagent Preparation section.

D. REAGENT PREPARATION

Bring all reagents to room temperature before use.

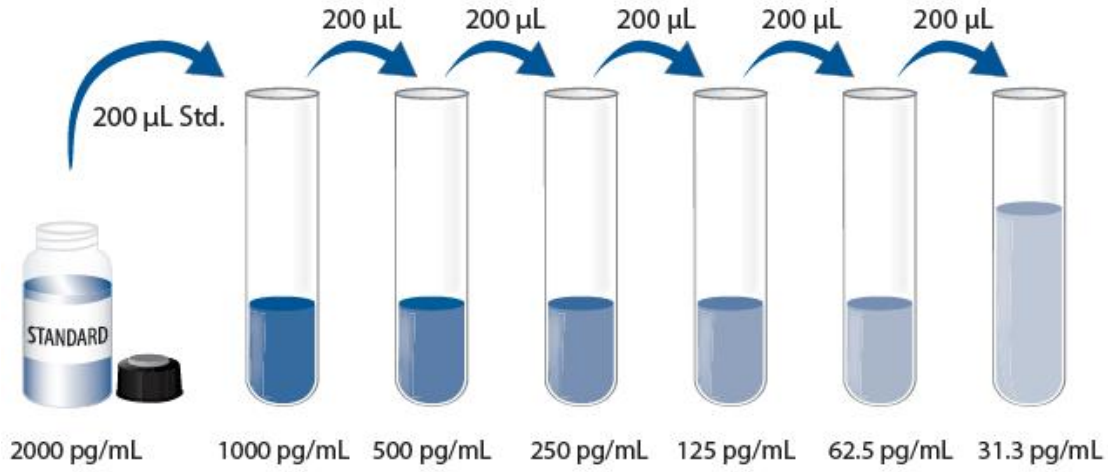
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

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, working standards, 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/urine samples)* or Assay Diluent RD1-73 *(for serum/platelet-poor plasma samples*) to each well.
4. Add 50 µL of Standard, and activated 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 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 TMB Substrate 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 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-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.

VIII. RERERENCES

1. Derynck, R. *et al*. (1986) J. Biol. Chem. 261:4377.

2. Padgett, R.W. and G.I. Patterson (2001) Developmental Cell 1:343.

3. Cox, D.A. and T. Maurer (1997) Clin. Immunol. Immunopathol. 83:25.

4. Ruscetti, F.W. and S.H. Bartelmez (2001) Int. J. Hematol. 74:18.

5. Gleizes, P-E. *et al*. (1997) Stem Cells 15:190.

6. Dubois, C.M. *et al*. (1995) J. Biol. Chem. 270:10618.

7. Khalil, N. (1999) Microbes Infect. 1:1255.

8. Clark, D.A. and R. Coker (1998) Int. J. Biochem. Cell Biol. 30:293.

9. Koli, K. *et al*. (2001) Microsc. Res. Tech. 52:354.

10. Mangasser-Stephan, K. and A.M. Gressner (1999) Cell Tissue Res. 297:363.

11. Qian, S.W. *et al*. (1990) Nucleic Acids Res. 18:3059.

12. Genbank Accession #: AAL87199.

13. Derynck, R. *et al*. (1985) Nature 316:701.

14. Manning, A.M. *et al*. (1995) Gene 155:307.

15. Kondaiah, P. *et al*. (1988) J. Biol. Chem. 263:18313.

16. Scarozza, A.M. *et al*. (1998) Cytokine 10:851.

17. Miller, D.A. *et al*. (1989) Mol. Endocrinol. 3:1108.

18. Denhez, F. *et al*. (1990) Growth Factors 3:139.

19. Derynck, R. and X-H. Feng (1997) Biochim. Biophys. Acta 1333:F105.

20. Ten Dijke, P. *et al*. (1996) Curr. Opin. Cell. Biol. 8:139.

21. Lawler, S. *et al*. (1994) Development 120:165.

22. Susuki, A. *et al*. (1994) Biochem. Biophys. Res. Commun. 198:1063.

23. Lopez-Casillas, F. *et al*. (1994) J. Cell Biol. 124:557.

24. St. Jacques, S. *et al*. (1994) Endocrinology 134:2645.

25. Barbara, N.P. *et al*. (1999) J. Biol. Chem. 274:584.

26. Ma, X. *et al*. (2000) Arterioscler. Thromb. Vasc. Biol. 20:2546.

27. Lux, A. *et al*. (1999) J. Biol. Chem. 274:9984.

28. Stavnezer, J. (1995) J. Immunol. 155:1647.

29. Sato, K. *et al*. (2000) J. Immunol. 164:2285.

30. Wahl, S.M. *et al*. (2000) Cytokine Growth Factor Rev. 11:71.

31. Ashcroft, G.S. and A.B. Roberts (2000) Cytokine Growth Factor Rev. 11:125.

32. Matsunaga, S. *et al*. (1999) Int. J. Oncol. 14:1063.

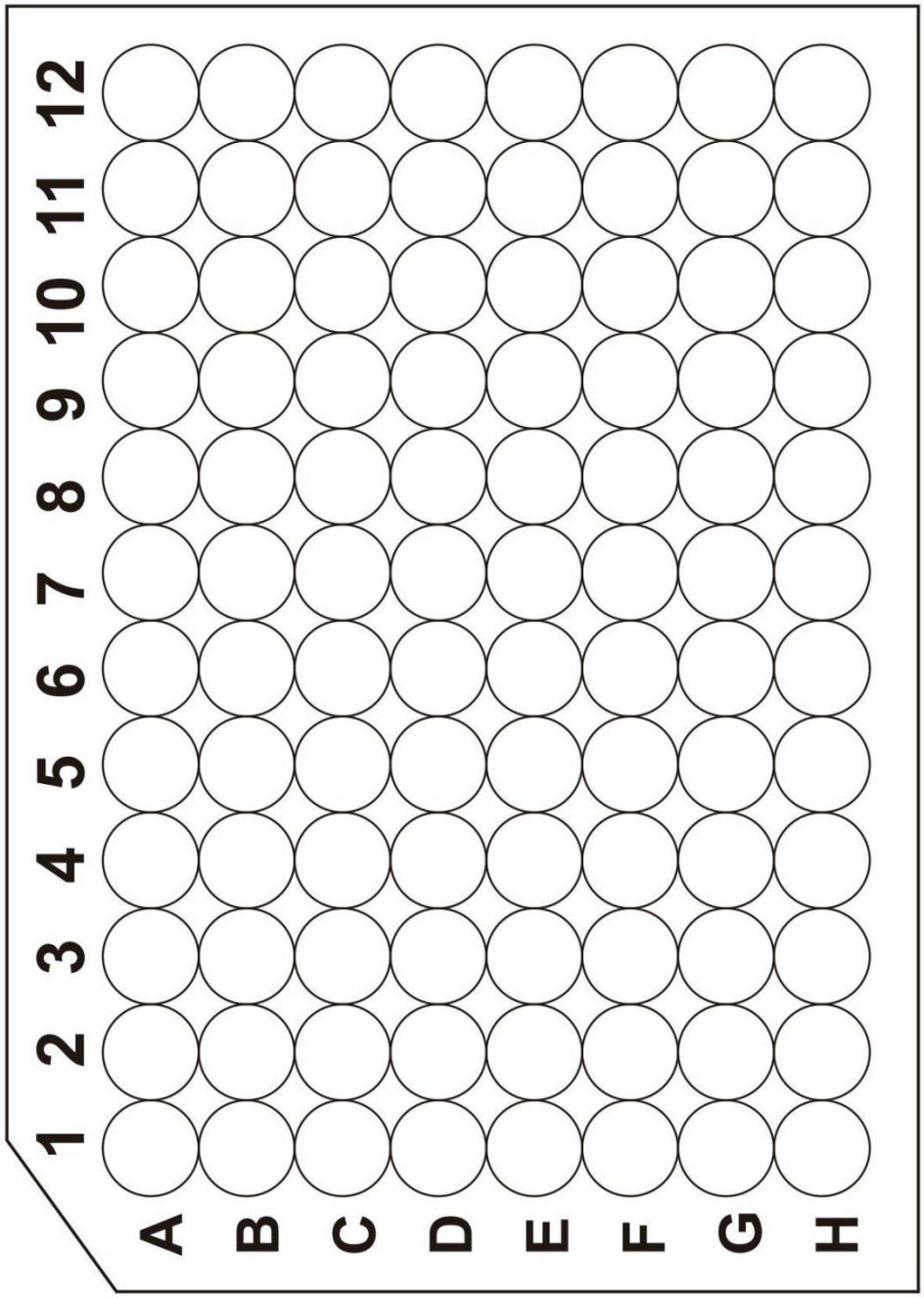
33. Mummery, C.L. *et al*. (1999) Int. J. Dev. Biol. 43:693.

34. Danielpour, D. *et al*. (1989) Growth Factors. 2:61.

35. Danielpour, D. *et al*. (1993) J. Immunol. Meth. 158:17.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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目录

I. 背景

人 TGF-β1 是一种 25 kDa 的二硫键连接的同源二聚体蛋白，参与多种关键的发育、免疫和稳态过程（1-4）。该分子由 390 个氨基酸(amino acid, aa)的前体合成，包含 23 aa 的信号序列、255 aa 的前肽区和 112 aa 的成熟片段。该分子的加工过程复杂，通常以潜伏形式分泌（5）。在释放前，前体原形式先被切除信号序列，随后其前肽区发生糖基化。糖基化过程包括甘露糖-6-磷酸残基的非典型连接。随后，弗林蛋白酶转化酶介导的前体激素切割，产生一个 80 kDa 的二硫键连接的前肽区（称为 LAP，即潜伏相关蛋白），以及一个 25 kDa 的二硫键连接的成熟片段（称为 TGF-β1）（6-8）。这两个独立的二硫键连接的多肽通过非共价相互作用结合，使 TGF-β1 失活。虽然这种 80K:25K 复合物可以直接分泌，但效率不高。为了促进分泌及细胞外储存，第三个 200 kDa 的组分，称为 LTBP，通过共价键连接到两条 LAP 多肽链其中一条的 N 末端。这促进了分泌以及随后在细胞外基质中的储存（9, 10）。分泌后，TGF-β1 通过 LTBP 共价连接到 ECM 上。该复合物随后被蛋白酶切割并释放，暴露出 LAP 上的甘露糖残基。据推测，暴露的 LAP 甘露糖残基现在能够与细胞表面的 IGF-II R 结合，在此发生解离事件，破坏 LAP-TGF-β1 复合物。这导致具有活性的同源二聚体 TGF-β1 的释放（7, 10）。成熟小鼠 TGF-β1 与大鼠和棉鼠 TGF-β1 的氨基酸序列同一性为 100%（11, 12），与人、犬和猪 TGF-β1 的氨基酸序列同一性为 99%（13-15），与豚鼠 TGF-β1 的氨基酸序列同一性为 97%（16）。相对于小鼠 TGF-β2 和β3，成熟小鼠 TGF-β1 的氨基酸序列同一性分别为 72%和 78%（17, 18）。

TGF-β1 传统的高亲和力受体是由跨膜丝氨酸/苏氨酸激酶组成的异源复合物。涉及两种类型：一种是组成型磷酸化的、与配体结合的 80 kDa 糖蛋白，称为 TGF-β1 RII；另一种是信号传导的、不结合配体的 55 kDa 糖蛋白，称为 TGF-β RI/ALK-5（19-22）。研究表明，TGF-β1 首先与 TGF-β1 RII 结合，然后启动 TGF-β1 RI 的交叉磷酸化，最终实现信号转导。还存在第三种 TGF-β受体，称为 TGF-β1 RIII，它可以是 250 kDa 的蛋白聚糖（称为 betaglycan），或者是 180 kDa 的糖蛋白（称为 endoglin/CD105）（23, 24）。有人提出 TGF-β1 RIII 捕获 TGF-β并将其“传递”给 TGF-β1 RII（20）。这可能适用于 betaglycan，但不适用于 endoglin。Endoglin 本身不结合 TGF-β；只有在 TGF-β1 RII 结合配体的背景下才能结合。证据表明，endoglin 可能不是“传递”配体，而是实际进入受体复合物并调节 TGF-β的下游信号传导（25, 26）。最后，尽管传统上认为 ALK-5 是 TGF-β1 唯一的 I 型信号受体，但 ALK-1 也可能作为条件依赖性的 I 型 TGF-β受体（27）。

TGF-β1 具有广泛的活性。在免疫反应过程中，TGF-β1 通过优先诱导小鼠和人类的 IgA 产生来影响抗体产生（28）。它还通过改变趋化因子受体的表达来调节树突状细胞的趋化性（29）。最后，它可以通过抑制巨噬细胞活性和促炎细胞因子分泌来下调炎症反应（30）。在伤口愈合过程中，TGF-β1 从活化的血小板中释放。这种局部来源的 TGF-β1 对成纤维细胞（诱导基质合成）、单核细胞（诱导促炎介质和生长因子分泌）以及角质形成细胞（可能通过下调其自身的信号通路来促进角质形成细胞增殖）具有显著的刺激作用（31）。最后，在发育过程中，TGF-β1 可能在软骨内骨化中发挥作用，其缺失会导致严重的卵黄囊血管生成和造血功能缺陷（32, 33）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗TGF-β1抗体包被于微孔板上，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TGF-β1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液、人/小鼠/大鼠/猪/犬血清、人尿液以及人/小鼠/大鼠/猪/犬去血小板血浆。
- 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）

已知浓度的三个样本，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。

细胞培养上清液

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	317	683	1271	312	657	1184
标准差	11.3	42.2	31.1	26.1	46.6	92.3
CV%	3.6	6.2	2.4	8.4	7.4	7.8

血清/血浆

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	309	708	1072	303	622	1092
标准差	15.3	40.0	44.3	21.2	47.2	70.0
CV%	5.0	5.6	4.1	7.0	7.6	6.4

B. 回收率

在活化的样本中掺入检测范围内不同水平的TGF-β1，测定其回收率。

样品类型	平均回收率（%）	范围（%）
人尿液(n=4)	114	105-123
人去血小板 EDTA 血浆(n=4)	90	77-107
人去血小板肝素血浆(n=4)	90	83-98
培养基+ FBS (n=4)	103	80-125
无血清培养基(n=3)	107	96-117
猪去血小板 EDTA 血浆(n=4)	87	82-95
猪去血小板肝素血浆(n=4)	85	76-95

C. 灵敏度

对三十三种检测方法进行了评估，人/小鼠/大鼠/猪/犬TGF-β1的最低检测剂量（MDD）范围为0.889-5.50 pg/mL，平均MDD为2.38 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TGF-β1蛋白所校正。

本试剂盒对NIBSC/WHO TGF-β1国际标准品89/514（人源，rDNA衍生）进行了评估。参考品89/514的剂量反应曲线与Valukine™标准曲线平行。若要将使用Valukine人/小鼠/大鼠/猪/犬TGF-β1试剂盒获得的样本数值近似转换为NIBSC/WHO 89/514单位，请使用以下公式：

细胞培养上清液/尿液 - NIBSC/WHO 89/514近似值(IU/mL) = 0.0159 × Valukine人/小鼠/大鼠/猪/犬TGF-β1数值(pg/mL)

血清/去血小板血浆 - NIBSC/WHO 89/514近似值(IU/mL) = 0.0171 × Valukine人/小鼠/大鼠/猪/犬TGF-β1数值 (pg/mL)

注意： *基于2021年5月生成的数据。*

E. 线性

不同的活化样本中掺入高浓度的TGF-β1，然后用标准品稀释液（1×）将样本稀释到检测范围内，测定其线性。

人类样本		细胞培养上清液（n=4）	血清*（n=4）	尿液（n=4）	去血小板	
					EDTA血浆*（n=4）	肝素血浆*（n=4）
1:2	平均预估百分比（%）	100	97	93	105	105
	范围（%）	90-114	95-102	92-94	103-107	102-111
1:4	平均预估百分比（%）	93	97	88	110	107
	范围（%）	82-117	94-104	83-91	108-113	103-112
1:8	平均预估百分比（%）	93	95	88	112	109
	范围（%）	76-121	93-100	83-92	111-112	102-116
1:16	平均预估百分比（%）	100	97	92	120	118
	范围（%）	85-124	92-104	86-101	115-124	110-125

*样本在活化后进行稀释。请参见样本活化步骤。

小鼠样本		细胞培养上清液*（n=4）	血清*（n=4）	去血小板	
				EDTA血浆*（n=4）	肝素血浆*（n=4）
1:2	平均预估百分比（%）	97	100	104	105
	范围（%）	91-102	92-105	90-115	103-111
1:4	平均预估百分比（%）	90	100	106	107
	范围（%）	86-96	92-104	95-115	102-113
1:8	平均预估百分比（%）	86	97	101	111
	范围（%）	79-95	91-101	88-110	100-119
1:16	平均预估百分比（%）	85	96	99	109
	范围（%）	79-94	87-101	88-110	93-117

*样本在活化后进行稀释。请参见样本活化步骤。

大鼠样本		细胞培养上清液*（n=4）	血清*（n=4）	去血小板	
				EDTA血浆*（n=4）	肝素血浆*（n=4）
1:2	平均预估百分比（%）	107	100	102	104
	范围（%）	107-108	93-104	99-109	101-108
1:4	平均预估百分比（%）	101	102	101	106
	范围（%）	100-103	99-107	96-104	104-110
1:8	平均预估百分比（%）	97	100	103	107
	范围（%）	96-97	96-103	99-109	103-111
1:16	平均预估百分比（%）	97	101	104	106
	范围（%）	96-98	96-108	97-115	97-113

*样本在活化后进行稀释。请参见样本活化步骤。

猪样本		细胞培养上清液*（n=4）	血清*（n=4）	去血小板	
				EDTA血浆*（n=5）	肝素血浆*（n=4）
1:2	平均预估百分比（%）	97	103	106	110
	范围（%）	88-106	100-106	102-111	108-111
1:4	平均预估百分比（%）	92	110	112	100
	范围（%）	81-102	97-106	109-111	108-115
1:8	平均预估百分比（%）	94	99	112	117
	范围（%）	81-103	95-104	110-114	108-124
1:16	平均预估百分比（%）	97	104	117	119
	范围（%）	86-106	97-109	115-118	109-124

*样本在活化后进行稀释。请参见样本活化步骤。

犬样本		细胞培养上清液*（n=3）	血清*（n=4）	去血小板	
				EDTA血浆*（n=4）	肝素血浆*（n=4）
1:2	平均预估百分比（%）	99	100	98	100
	范围（%）	91-104	96-102	91-103	96-105
1:4	平均预估百分比（%）	99	99	94	96
	范围（%）	96-102	95-103	87-98	87-107
1:8	平均预估百分比（%）	100	100	92	98
	范围（%）	93-109	97-103	87-95	88-106
1:16	平均预估百分比（%）	104	100	95	101
	范围（%）	95-120	95-107	86-105	96-106

*样本在活化后进行稀释。请参见样本活化步骤。

F. 样本预值

血清/血浆/尿液 - 此方法测定样本中TGF-β1含量。

人类样品	平均值（pg/mL）	范围（pg/Ml）	标准差（pg/mL）
血清(n=31)	51,640	32,091-95,147	11,901
去血小板 EDTA 血浆(n=31)	2377	1414-4641	729
去血小板肝素血浆(n=31)	2383	1445-3653	561
尿液* (n=10)	65.9	ND-108	—

*仅有 40%的尿液样本检测到可测水平（> 31.3 pg/mL）。

ND = 未检出。

小鼠样本	平均值（pg/mL）	范围（pg/MI）	标准差（pg/mL）
血清(n=10)	97,352	78,751-114,130	12,623
去血小板 EDTA 血浆(n=10)	41,763	16,577-68,505	16,887
去血小板肝素血浆(n=10)	41,101	18,195-66,700	17,419

大鼠样本	平均值（pg/mL）	范围（pg/MI）	标准差（pg/mL）
血清(n=10)	68,108	43,721-89,138	14,343
去血小板 EDTA 血浆(n=5)	5833	4912-7820	1141
去血小板肝素血浆(n=5)	13,971	5274-26,114	8392

猪样本	平均值（pg/mL）	范围（pg/MI）	标准差（pg/mL）
血清(n=5)	17,597	13,481-23,914	4146
去血小板 EDTA 血浆(n=5)	1955	1530-2810	518
去血小板肝素血浆(n=5)	1567	1111-2475	551

犬样本	平均值（pg/mL）	范围（pg/MI）	标准差（pg/mL）
血清(n=10)	33,455	2193-79,590	19,795
去血小板 EDTA 血浆** (n=5)	2195	ND-2515	—
去血小板肝素血浆*** (n=5)	3763	ND-4278	—

**仅有 60%的 EDTA 血浆样本在要求的 40 倍稀释下检测到可测水平。五个样本中有两个的读数略低于标准曲线（< 31.3 pg/mL）。

***仅有80%的肝素血浆样本在要求的40倍稀释下检测到可测水平。五个样本中有一个的读数略

低于标准曲线（< 31.3 pg/mL）。

细胞培养上清：通过密度梯度离心法，使用 Ficoll-Paque Plus 从全血中分离人外周血单个核细胞（PBMC）。使用 MagCellect™人 $CD4^+$ T 细胞分离试剂盒（R&D Systems®，货号 # MAGH102）从 PBMC 中分离 $CD4^+$ T 细胞。细胞以 5×10^5个/mL 的密度接种，并使用 Excellerate™人 T 细胞扩增培养基（无动物源成分，R&D Systems，货号 # CCM030）进行培养。T 细胞未经处理，或使用 10 ng/mL GMP 重组人（rh）IL-7（R&D Systems，货号 # 207-GMP）、10 ng/mL GMP rhIL-15（R&D Systems，货号 # 247-GMP）处理，并通过其 T 细胞受体（TCR）和共刺激受体刺激 5 天。TCR 刺激通过每 mL 培养液加入 25 µL Cloudz™ CD3/28 颗粒（Cloudz T 细胞活化试剂盒 - CD3/CD28，R&D Systems，货号 # CLD001）实现。$CD4^+$ T 细胞在 37°C、5% CO_2 培养箱中维持 5 天。取等份细胞培养上清，检测 TGF-β1，测得结果为：未处理组 77.7 pg/mL，处理组 471 pg/mL。

人 PBMC 以 1×10^6个/mL 的密度接种，在含 10%胎牛血清（FBS）、2 mM L-谷氨酰胺、100 U/mL 青霉素和 100 µg/mL 链霉素的 RPMI 培养基中培养，且不作处理。取等份细胞培养上清，检测 TGF-β1，测得结果为 2854 pg/mL。

小鼠 EL-4 细胞在含 10% FBS、2 mM L-谷氨酰胺、100 U/mL 青霉素和 100 µg/mL 链霉素的高糖 DMEM 培养基中培养。随后用 10 ng/mL PMA 和 10 µg/mL PHA 处理细胞 24 小时。取等份细胞培养上清，检测 TGF-β1，测得结果为 2969 pg/mL。

从怀孕的 Sprague Dawley 大鼠中取出大鼠脾脏，匀浆后培养于含 10% FBS、2 mM L-谷氨酰胺、100 U/mL 青霉素和 100 µg/mL 链霉素的 DME 培养基中，在 37°C、5% CO_2 条件下培养。大鼠脾细胞用 50 ng/mL 重组大鼠 IL-2 和 5 µg/mL PHA 处理 3 天。取等份细胞培养上清，检测 TGF-β1，测得结果为 2716 pg/mL。

猪 PK-15 细胞在含 10% FBS、2 mM L-谷氨酰胺、100 U/mL 青霉素和 100 µg/mL 链霉素的 MEM 培养基中培养。细胞用 1 µg/mL LPS 处理 72 小时。取等份细胞培养上清，检测 TGF-β1，测得结果为 1631 pg/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。将以下因子用标准品稀释液（1×）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-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		重组小鼠	其他重组蛋白
Activin A	BMPR-IB	Activin RI	rat Agrin
Activin RIA	BMPR-II	Activin RIIA	zebrafish BMP-2
Activin RIIA	$Follistatin_{288}$	Activin RIB	amphibian TGF-β5
BMPR-IB	$Follistatin_{300}$	Activin RIIB	chinese hamster TGF-β1-LAP
Activin RIIB	$Follistatin_{315}$	BMP-3	
Agrin	GDF-5	BMP-3b	天然蛋白
BMP-1.1	GDF-7	BMP-4	porcine TGF-β2
BMP-2	GDF-9	BMP-6	
BMP-3	GDF-11	BMP-7	
BMP-3b	GDF-15	BMP-9	
BMP-4	Inhibin A	BMPR-IA	
BMP-5	Inhibin B	BMPR-IB	
BMP-6	LAP (TGF-β1)	BMPR-II	
BMP-7	TGF-α	$Follistatin_{288}$	
BMP-8a	TGF-β RI	Inhibin A	
BMP-8b	TGF-β RIII	TGF-β RI	
BMP-10	TGF-β3	TGF-β RIII	
BMP-15			
BMPR-IA			

交叉反应：交叉反应检测到与下列因子存在交叉反应。

重组蛋白	检测稀释液	
	RD1-21	**RD1-73**
Human BMP-9	<1%	<1%
Mouse BMP-10	<1%	<1%
Human Latent TGF-β1	<1%	<1%
Activated Human Latent TGF-β1	15%	15%
Equine TGF-β1	3%	<1%
Human TGF-β1.2	14%	12%
Human TGF-β2	13%	12%

干扰：干扰实验检测到重组人TGF-β RII和重组小鼠TGF-β RII存在显著干扰。

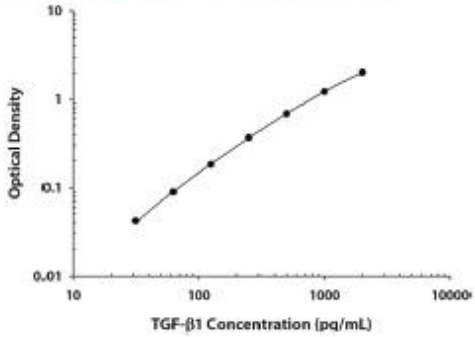
人源或小鼠源 TGF-β RII (ng/mL)	**TGF-β1 (pg/mL)**	**存在人TGF-β RII时检测到的TGF-β1百分比(%)**		**存在小鼠TGF-β RII时检测到的TGF-β1百分比(%)**	
		RD1-21	**RD1-73**	**RD1-21**	**RD1-73**
0	500	100	100	100	100
1.56	500	98	99	90	93
3.13	500	93	95	85	88
6.25	500	82	86	77	79
12.5	500	63	66	69	67
25	500	42	37	60	55
50	500	28	18	52	40

IV. 实验

标准曲线实例

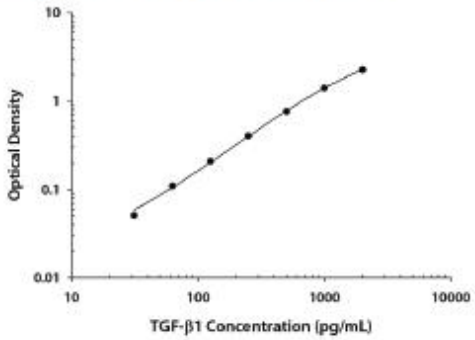
该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

CELL CULTURE SUPERNATE/URINE ASSAY



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

SERUM/PLATELET-POOR PLASMA ASSAY



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

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TGF-β1检测抗体	1瓶
TGF-β1 Standard	重组TGF-β1（冻干）。参考瓶身标签进行重溶	2瓶
Calibrator Diluent（2×） RD6-11	浓缩的标准品稀释液（2×）用于稀释标准品和样本	1瓶
Assay Diluent RD1-21	检测液，*适用于细胞培养上清液/尿液样本*	1瓶
Assay Diluent RD1-73	检测液，*适用于血清/去血小板血浆样本*	1瓶
Wash Buffer Concentrate（25×）	浓缩洗涤液（25×）	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

<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="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="6">2-8℃储存，最多30天*。</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>检测液 RD1-21</td></tr>
<tr><td>检测液 RD1-73</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>2-8℃储存，最多30天*
每次检测请使用新的标准品。用后即弃。</td></tr>
<tr><td>标准品稀释液（2×）
RD6-11</td><td>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。</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；
- 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、面部及衣服的防护。

VI. 实验前准备

A. 样品收集及储存

下述样本采集和储存条件仅作为一般性指南。样本稳定性尚未经过评估。活化后的样本必须立即检测。请勿冷冻活化后的样本。

细胞培养上清液 - 颗粒物应离心去除；立刻检测样本（参见活化步骤）或分装，冻存于≤-20℃冰箱内，避免反复冻融。样本可能需要用稀释液（1×）稀释。

注意： *用于制备细胞培养基的动物血清可能含有高水平的潜伏型TGF-β1。为获得最佳结果，在检测TGF-β1产量时，请勿使用动物血清进行细胞培养。如果将动物血清作为培养基补充物使用，应采取预防措施制备适当的对照品，并在免疫分析中检测该对照品，以确定TGF-β1的基础浓度。*

人血清样本 - 用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后1000 × g离心15分钟。吸取血清样本之后即刻用于检测（参见活化步骤），或者分装，≤-20℃贮存备用。避免反复冻融。样本可能需要用标准品稀释液（1×）稀释。

非人血清 - 使血液样本在室温下凝集2小时，然后以2000 × g离心20分钟。取出血清，立即检测（参见活化步骤），或者分装，≤-20℃贮存备用。避免反复冻融。

人尿液 - 无菌采集每日首次尿液（中段尿），直接排入无菌容器中。离心去除颗粒物。立即检测（参见活化步骤），或者分装，≤-20℃贮存备用。避免反复冻融。

注意： *未经活化的纯尿液样本在储存（冷冻或冷藏）的头24小时内，TGF-β1浓度会下降。应注意确保样本在相同的储存条件和储存时长下进行检测。*

去血小板血浆* - 在冰上使用EDTA或肝素作为抗凝剂采集血浆。采集后30分钟内以1000 × g离心20分钟。建议在2-8 °C下以10,000 × g额外离心10分钟，以完全去除血小板。立即检测（参见活化步骤），或者分装，≤-20℃贮存备用。避免反复冻融。

注意： *枸橼酸盐血浆尚未经验证可用于本检测。*

***TGF-β1存在于血小板颗粒中，并在血小板活化时释放。因此，要检测TGF-β1的循环水平，应采集去血小板血浆进行测定。需注意，许多血浆制备方案（包括临床和实验室标准协会（CLSI）推荐的程序）都无法从血浆中完全去除血小板。这将对检测血小板所含且经血小板活化释放的因子产生可变且不可重复的结果。推荐的血浆采集方案旨在最大限度地减少血小板脱颗粒。然而，即使最佳的血浆采集方法有时也可能导致一定程度的血小板脱颗粒，建议在TGF-β1水平升高的样本中测定血小板脱颗粒的标志物。**

B. 样本活化

活化试剂的制备

为了将潜伏型TGF-β1活化为免疫反应形式，请准备以下用于酸活化和中和的溶液。这些溶液可在室温下储存于聚丙烯瓶中，最长可保存一个月。

警告：在制备和使用这些试剂时，请穿戴防护服和护目镜。使用前请参阅相应的安全数据表（SDS）。

1 N HCl（100 mL） - 在91.67 mL去离子水中缓慢加入8.33 mL 12 N HCl，充分混匀。

1.2 N NaOH / 0.5 M HEPES（100 mL） - 在75 mL去离子水中缓慢加入12 mL 10 N NaOH，充分混匀。加入11.9 g HEPES，混匀。用去离子水定容至100 mL。

对于每批新的酸化和中和试剂，需测量中和后几个代表性样品的pH值，确保其在7.2-7.6范围内。根据需要调整中和试剂的体积及相应的稀释倍数。

TGF-β1样本活化操作步骤

为了将潜伏型TGF-β1活化为可通过TGF-β1免疫分析法检测的免疫反应性TGF-β1，请按照以下活化步骤进行操作。检测样本需经过中和反应（pH 7.2-7.6），使用聚丙烯试管。

注意： ***请勿活化试剂盒中的标准品。****标准品中已含有活性的重组TGF-β1。*

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
检测样本前使用建议标准品稀释液稀释（1×）。建议的稀释倍数请参见下文。	检测样本前使用建议标准品稀释液稀释（1×）。建议的稀释倍数请参见下文。
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

C. 样本准备工作

细胞培养上清样本（人及非人） - 直接检测原液，但如果内源性水平较高可能需要进行稀释（直接检测原液时，样本的最终稀释倍数为1.4）。最佳稀释倍数应由最终用户确定。

人血清样本 - 需用标准品稀释液（1×）进行40倍稀释†。建议的40倍稀释方法：10 µL活化样本 + 390 µL标准品稀释液（1×）（样本最终稀释倍数为60）。最佳稀释倍数应由最终用户确定。

人尿液样本 - 直接检测原液（样本最终稀释倍数为1.4）。最佳稀释倍数应由最终用户确定。

人去血小板血浆*样本 - 需用标准品稀释液（1×）进行8倍稀释†。建议的8倍稀释方法：25 µL活化样本 + 175 µL标准品稀释液（1×）（样本最终稀释倍数为12）。最佳稀释倍数应由最终用户确定。

小鼠和大鼠血清/去血小板血浆*样本 - 需用标准品稀释液（1×）进行60倍稀释†。建议的60倍稀释方法：10 µL活化样本 + 590 µL标准品稀释液（1×）（样本最终稀释倍数为90）。最佳稀释倍数应由最终用户确定。

猪血清样本 - 需用标准品稀释液（1×）进行15倍稀释。建议的15倍稀释方法：10 µL活化样本 + 140 µL标准品稀释液（1×）（样本最终稀释倍数为22.5）。最佳稀释倍数应由最终用户确定。

猪去血小板血浆*样本 - 需用标准品稀释液（1×）进行5倍稀释。建议的5倍稀释方法：40 µL活化样本 + 160 µL标准品稀释液（1×）（样本最终稀释倍数为7.5）。最佳稀释倍数应由最终用户确定。

犬血清和去血小板血浆*样本 - 需用标准品稀释液（1×）进行40倍稀释。建议的40倍稀释方法：10 µL活化样本 + 390 µL标准品稀释液（1×）（样本最终稀释倍数为60）。最佳稀释倍数应由最终用户确定。

*根据血小板污染程度不同，可能需要不同的稀释倍数。

†参见试剂配制部分。

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

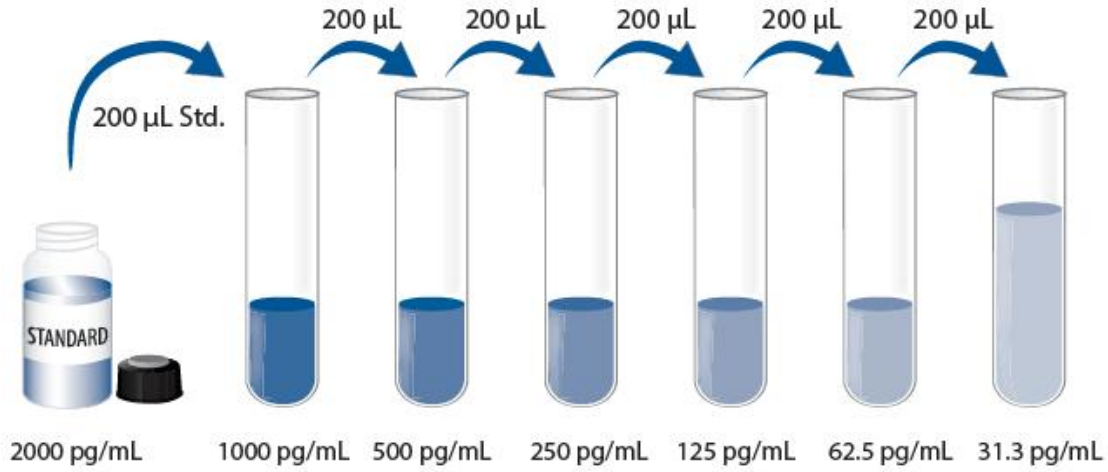
去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

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、标准品和活化后的样本；
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RD1-21（*用于细胞培养上清/尿液样本*）或检测稀释液RD1-73（*用于血清/去血小板血浆样本*）；
4. 分别将不同浓度标准品或活化后的样本加入相应孔中，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-β1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

1. Derynck, R. et al. (1986) J. Biol. Chem. 261:4377.

2. Padgett, R.W. and G.I. Patterson (2001) Developmental Cell 1:343.

3. Cox, D.A. and T. Maurer (1997) Clin. Immunol. Immunopathol. 83:25.

4. Ruscetti, F.W. and S.H. Bartelmez (2001) Int. J. Hematol. 74:18.

5. Gleizes, P-E. et al. (1997) Stem Cells 15:190.

6. Dubois, C.M. et al. (1995) J. Biol. Chem. 270:10618.

7. Khalil, N. (1999) Microbes Infect. 1:1255.

8. Clark, D.A. and R. Coker (1998) Int. J. Biochem. Cell Biol. 30:293.

9. Koli, K. et al. (2001) Microsc. Res. Tech. 52:354.

10. Mangasser-Stephan, K. and A.M. Gressner (1999) Cell Tissue Res. 297:363.

11. Qian, S.W. et al. (1990) Nucleic Acids Res. 18:3059.

12. Genbank Accession #: AAL87199.

13. Derynck, R. et al. (1985) Nature 316:701.

14. Manning, A.M. et al. (1995) Gene 155:307.

15. Kondaiah, P. et al. (1988) J. Biol. Chem. 263:18313.

16. Scarozza, A.M. et al. (1998) Cytokine 10:851.

17. Miller, D.A. et al. (1989) Mol. Endocrinol. 3:1108.

18. Denhez, F. et al. (1990) Growth Factors 3:139.

19. Derynck, R. and X-H. Feng (1997) Biochim. Biophys. Acta 1333:F105.

20. Ten Dijke, P. et al. (1996) Curr. Opin. Cell. Biol. 8:139.

21. Lawler, S. et al. (1994) Development 120:165.

22. Susuki, A. et al. (1994) Biochem. Biophys. Res. Commun. 198:1063.

23. Lopez-Casillas, F. et al. (1994) J. Cell Biol. 124:557.

24. St. Jacques, S. et al. (1994) Endocrinology 134:2645.

25. Barbara, N.P. et al. (1999) J. Biol. Chem. 274:584.

26. Ma, X. et al. (2000) Arterioscler. Thromb. Vasc. Biol. 20:2546.

27. Lux, A. et al. (1999) J. Biol. Chem. 274:9984.

28. Stavnezer, J. (1995) J. Immunol. 155:1647.

29. Sato, K. et al. (2000) J. Immunol. 164:2285.

30. Wahl, S.M. et al. (2000) Cytokine Growth Factor Rev. 11:71.

31. Ashcroft, G.S. and A.B. Roberts (2000) Cytokine Growth Factor Rev. 11:125.

32. Matsunaga, S. et al. (1999) Int. J. Oncol. 14:1063.

33. Mummery, C.L. et al. (1999) Int. J. Dev. Biol. 43:693.

34. Danielpour, D. et al. (1989) Growth Factors. 2:61.

35. Danielpour, D. et al. (1993) J. Immunol. Meth. 158:17.

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