PRODUCT INFORMATION & MANUAL

Human/Mouse/Rat Activin A Valukine™ ELISA

Catalog Number: VAL203

For the quantitative determination of natural and recombinant human/mouse/rat Activin A 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 202411.1

TABLE OF CONTENTS

I. BACKGROUND

Activin and Inhibin, members of the TGF-β superfamily of cysteine knot cytokines, were originally purified from gonadal fluids as proteins that stimulated or inhibited, respectively, follicle stimulating hormone (FSH) release from the pituitary. They have since been shown to be involved in a wide range of biological processes including tissue morphogenesis and repair, fibrosis, inflammation, neural development, hematopoiesis, reproductive system function, and carcinogenesis (1-7).

Activin and Inhibin are produced as precursor proteins. Their amino terminal propeptides are proteolytically cleaved and facilitate formation of disulfide-linked dimers of the bioactive proteins (8-10). Activins are non-glycosylated homodimers or heterodimers of various β subunits (βA, βB, βC, and βE in mammals), while Inhibins are heterodimers of a unique α subunit and one of the β subunits. Activin A is a homodimer of two βA chains. The βA subunit can also heterodimerize with a βB or βC subunit to form Activin AB and Activin AC, respectively (11, 12). The 14 kDa mature human βA chain shares 100% amino acid sequence identity with bovine, feline, mouse, porcine, and rat βA. The βA chain is expressed by many cell types including fibroblasts and keratinocytes (13), vascular smooth muscle cells (14), epithelial and endothelial cells (14, 15), hepatocytes (16), osteoclasts and chondrocytes (17, 18), monocytes and macrophages (14, 19), neurons (20), somatic cells of the ovary and testes (21), and anterior pituitary gonadotrophs (22).

Activin A exerts its biological activities by binding to the type 2 serine/threonine kinase Activin RIIA which then noncovalently associates with the type 1 serine/threonine kinase Activin RIB/ALK-4 (7, 23, 24). Signaling through this receptor complex leads to Smad activation and regulation of activin-responsive gene transcription (7, 24). The bioactivity of Activin A is regulated by a variety of mechanisms (24). BAMBI, TGF-β RIII, and Cripto are cell-associated molecules that function as decoy receptors or limit the ability of Activin A to induce receptor complex assembly (25-27). The intracellular formation of Activin A can be prevented by the incorporation of the βA subunit into Activin AC or Inhibin A (3, 11, 12). The bioavailability of Activin A is restricted by its incorporation into inactive complexes with α2-Macroglobulin, Follistatin, and FLRG (28-30).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs a 3-step quantitative sandwich enzyme immunoassay technique. The capture antibody specific for human/mouse/rat Activin A is biotinylated and bound to streptavidin-coated plates. The plates are washed and assay diluent, standards, and samples are pipetted into the wells and any human/mouse/rat Activin A present is bound by the immobilized antibody. After washing away any unbound substances, an HRP-conjugate specific for the human/mouse/rat βA subunit is added to the wells. Following a wash to remove any unbound conjugate, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human/mouse/rat Activin A bound. 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, human/mouse/rat serum, human/mouse/rat plasma and human/mouse/rat saliva.
- 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 RD5-54 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 forty separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Mean (pg/mL)	101	295	498	106	308	529
Standard Deviation	4.2	12.5	21.9	8.4	15.5	24.8
CV%	4.2	4.2	4.4	7.9	5.0	4.7

B. RECOVERY

The recovery of human/mouse/rat Activin A spiked to three different levels in human samples throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture media (n=4)	105	93-114
Human Serum* (n=4)	109	100-115
Human EDTA plasma* (n=4)	110	95-115
Human Heparin plasma* (n=4)	107	99-113

*Samples were diluted prior to assay.

C. SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human/mouse/rat Activin A ranged from 0.75-7.85 pg/mL. The mean MDD was 3.67 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 a highly purified CHO cell-expressed recombinant human/mouse/rat Activin A produced at R&D Systems.

The NIBSC/WHO Activin A First Reference Reagent Preparation (91/626), which is intended as a potency standard, was evaluated in this kit. The dose response curve of this standard parallels the Valukine Activin A standard curve. To convert sample values obtained with the Valukine Human/Mouse/Rat Activin A assay to approximate NIBSC 91/626 nominally assigned mass values, use the equation below.

NIBSC (91/626) approximate value (mU/mL) = 1.185 × Valukine Activin A value (pg/mL)

E. LINEARITY

To assess the linearity of the assay, human samples were containing or spiked with high concentrations of human/mouse/rat Activin A and diluted with Calibrator Diluent RD5-54 to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media* (n=4)	Human serum* (n=4)	Human EDTA plasma* (n=4)	Human heparin* plasma (n=4)	Human Saliva* (n=4)
1:2	Average % of Expected	99	107	102	109	103
	Range (%)	93-106	105-111	101-104	104-112	99-109
1:4	Average % of Expected	97	108	104	112	91
	Range (%)	90-103	101-115	100-111	109-114	82-98
1:8	Average % of Expected	96	110	107	112	93
	Range (%)	89-108	107-119	102-116	103-118	____
1:16	Average % of Expected	102	101	98	105	____
	Range (%)	96-108	94-116	88-114	88-120	____

*Samples were diluted prior to assay.

F. SAMPLE VALUES

Human/Mouse/Rat Serum/Plasma/Saliva – Samples were evaluated for the presence of human/mouse/rat Activin A in this assay. No medical histories were available for the donors used in this study.

Human Samples	Mean (pg/mL)	Range (pg/mL)	Standard Devation (pg/mL)
Human Serum (n=35)	352	142-753	129
Human EDTA plasma (n=35)	319	115-665	124
Human Heparin plasma (n=35)	316	111-695	126

Human Samples	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Human Saliva (n=13)	192	85	ND-428

ND=Non-detectable

Mouse Samples	Mean (pg/mL)	Range (pg/mL)	Standard Devation (pg/mL)
Mouse Serum (n=20)	197	78.1-352	93.1
Mouse EDTA plasma (n=20)	254	136-471	84.2
Mouse Heparin plasma (n=20)	188	130-272	46.0

Rat Samples	Mean (pg/mL)	Range (pg/mL)	Standard Devation (pg/mL)
Rat Serum (n=8)	306	109-1466	469
Rat EDTA plasma (n=9)	214	97.7-312	63.9
Rat Heparin plasma (n=7)	276	210-342	42.9

Cell Culture Supernates:

Note: *Cell culture supernates were not corrected for the presence of fetal bovine serum.*

IMR-90 human fetal lung fibroblasts were cultured in MEM with 10% fetal bovine serum until confluent. An aliquot was removed, assayed for human/mouse/rat Activin A, and measured 5608 pg/mL.

WS-1 human fetal skin fibroblasts were cultured in MEM with 10% fetal bovine serum, NEAA, and 2 mM L-glutamine until confluent. Cells were then stimulated for an additional 24 hours with 100 ng/mL LPS, 10 ng/mL recombinant human IL-1β, or 10 ng/mL recombinant human TNF-α. Aliquots were removed and assayed for levels of human/mouse/rat Activin A.

Stimulant	Day 1 (pg/mL)
LPS	8489
IL-1β	21,033
TNF-α	21,098

CMT-93 mouse rectal carcinoma cells were cultured in MEM with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot was removed, assayed for human/mouse/rat Activin A, and measured 655 pg/mL.

ST-2 mouse bone marrow-derived stromal cells were cultured in DMEM with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot was removed, assayed for human/mouse/rat Activin A, and measured 3156 pg/mL.

LL/2 mouse Lewis lung carcinoma cells were cultured in DMEM with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot was removed, assayed for human/mouse/rat Activin A, and measured 44.3

pg/mL.

C6 rat glioma cells were cultured in DMEM with 10% fetal bovine serum and 2 mM L-glutamine until confluent. An aliquot was removed, assayed for human/mouse/rat Activin A, and measured 1559 pg/mL.

C58(NT)D rat thymic lymphoma cells were cultured in DMEM with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 4.5 g/L glucose until confluent. An aliquot was removed, assayed for human/mouse/rat Activin A, and measured 21.0 pg/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant human/mouse/rat Activin A.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-54 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human/mouse/rat Activin A control were also assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:		**Recombinant mouse:**	**Recombinant rat:**
Activin AC Heterodimer	BMP-15	Activin C	Agrin
Activin B	Cripto	Activin RIB	ALK-7
Activin C	DAN	Activin RIIB	MIS
Activin RIA	Endoglin	ALK-1	**Other recombinants:**
Activin RIB	Follistatin 288	BAMBI	zebrafish BMP-2
Activin RIIA	Follistatin 300	BMPRIA	porcine TGF-β2
Activin RIIB	Follistatin 315	BMPRIB	amphibian TGF-β5
ALK-1	Inhibin B	BMP-3b/GDF-10	**Natural proteins:**
BAMBI	Lefty A	Cripto	porcine TGF-β1
BMPRII	MIS	DAN	
BMP-1/PCP	Osteoactivin	Endoglin	
BMP-2	LAP	Lefty	

BMP-3	TGF-α	MIS	
BMP-3b/GDF-10	TGF-β1	Noggin	
BMP-4	TGF-β1.2	Osteoactivin	
BMP-5	TGF-β2		
BMP-6	TGF-β3		
BMP-8b	TGF-βRII		
BMP-10	TGF-βRIII/betaglycan		

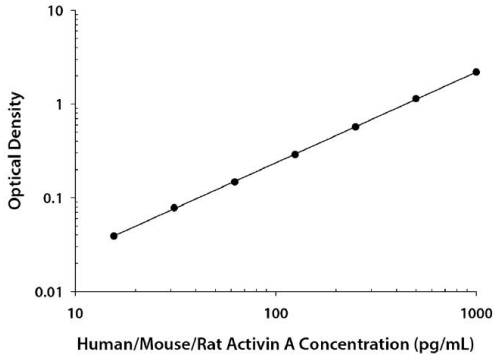
Recombinant human Inhibin A cross-reacts approximately 0.2% in this assay.

Recombinant human Activin AB cross-reacts approximately 0.45% in this assay.

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	0.051 0.051	0.051	—
15.6	0.089 0.090	0.090	0.039
31.3	0.127 0.130	0.129	0.078
62.5	0.196 0.199	0.198	0.147
125	0.330 0.350	0.340	0.289
250	0.601 0.639	0.620	0.569
500	1.180 1.199	1.190	1.139
1000	2.207 2.258	2.233	2.182

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Streptavidin Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with Streptavidin	1 plate
Activin A Biotinylated Antibody	An antibody specific for human/mouse/rat Activin A conjugated to biotin	1 vial
Activin A Conjugate	Solution of antibody against human/mouse/rat Activin A conjugated to horseradish peroxidase	1 vial
Activin A Standard	Recombinant human Activin A in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	1 vial
Assay Diluent RD1-98	A buffered protein base with blue dye	1 vial
Calibrator Diluent RD5-54	A buffered protein base used to dilute standard and samples	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	2 vials
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Sealers	Adhesive strip	8 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>Wash Buffer (1×)</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>Activin A Biotinylated Antibody</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Assay Diluent RD1-98</td></tr>
<tr><td>Calibrator Diluent RD5-54</td></tr>
<tr><td>Standard</td></tr>
<tr><td>TMB Substrate</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.
- 1000 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500±50 rpm
- Polypropylene test tubes for dilution of standards.

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.

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 RD5-54.

Note: *Culture media containing biotin, such as RPMI and McCoy's media, are not suitable for use in this assay. Animal serum used in the preparation of cell culture media may contain endogenous levels of Activin A. For best results, do not use animal serum for growth of cell cultures when assaying for Activin A 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 Activin A.*

Human 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 RD5-54.

Mouse Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 × 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 RD5-54.

Rat Serum - Allow blood samples to clot for 2 hours at room temperature before centrifugation for 30 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 RD5-54.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-54.

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

Grossly hemolyzed or lipemic samples are not suitable for use in this assay.

Saliva - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-54.

Note: *Saliva collector must not have any protein binding or filtering capabilities.*

B. SAMPLE PREPARATION

Human/Mouse/Rat serum, plasma, and saliva samples recommend at least a 2-fold dilution. A suggested 2-fold dilution is 150 µL of sample + 150 µL of Calibrator Diluent RD5-54. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

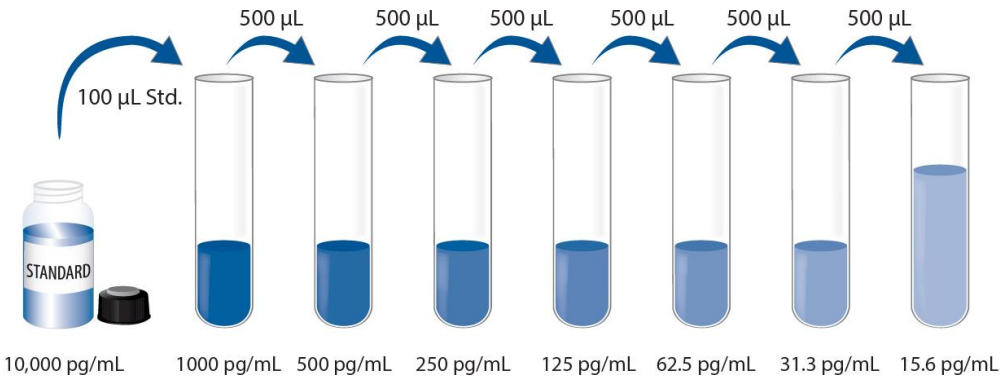
Note: *High concentrations of human/mouse/rat Activin A are found in human/mouse/rat saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 40 mL of Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 mL of Wash Buffer (1×).

Activin A Standard - Refer to the vial label for the reconstitution volume* Reconstitute the Activin A Standard with deionized or distilled water. This reconstitution produces a stock solution of 10000 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.

Pipette 900 µL of Calibrator Diluent RD5-54 into the 1000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-54 serves as the zero standard (0 pg/mL).



D. MICROPLATE PREPARATION

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Pipette 200 µL of the Activin A Biotinylated Antibody into all wells. Securely cover and incubate for 15 minutes at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500±50 rpm.
3. Aspirate each well and wash, repeating the process for a total of two 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.
4. Proceed to the Assay Procedure immediately after wash. Do not allow the wells to dry.

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.
- 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.
- 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 other reagents and samples to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.

Note: *High concentrations of human/mouse/rat Activin A are found in human/mouse/rat saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, microplate, and samples as directed in the previous sections.
2. Add 100 µL of Assay Diluent RD1-98 to each well.
3. Add 100 µL of standard and prepared sample per well. A plate layout is provided for a record of standards and samples assayed.
4. Cover with the adhesive strip provided. **Incubate for 3 hours at room temperature. on a horizontal orbital microplate shaker (0.12" orbit) set at 500±50 rpm.**
5. Aspirate each well and wash, repeating the process five times for a total of six 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 200 µL of Activin A Conjugate to each well. Cover with a new adhesive strip. **Incubate for 1 hour at room temperature on the shaker.**
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 50 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 (O.D.). Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the human/mouse/rat Activin A concentrations versus the log of the O.D. on a linear scale and the best fit line can be determined by regression analysis.

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

VIII. REFERENCES

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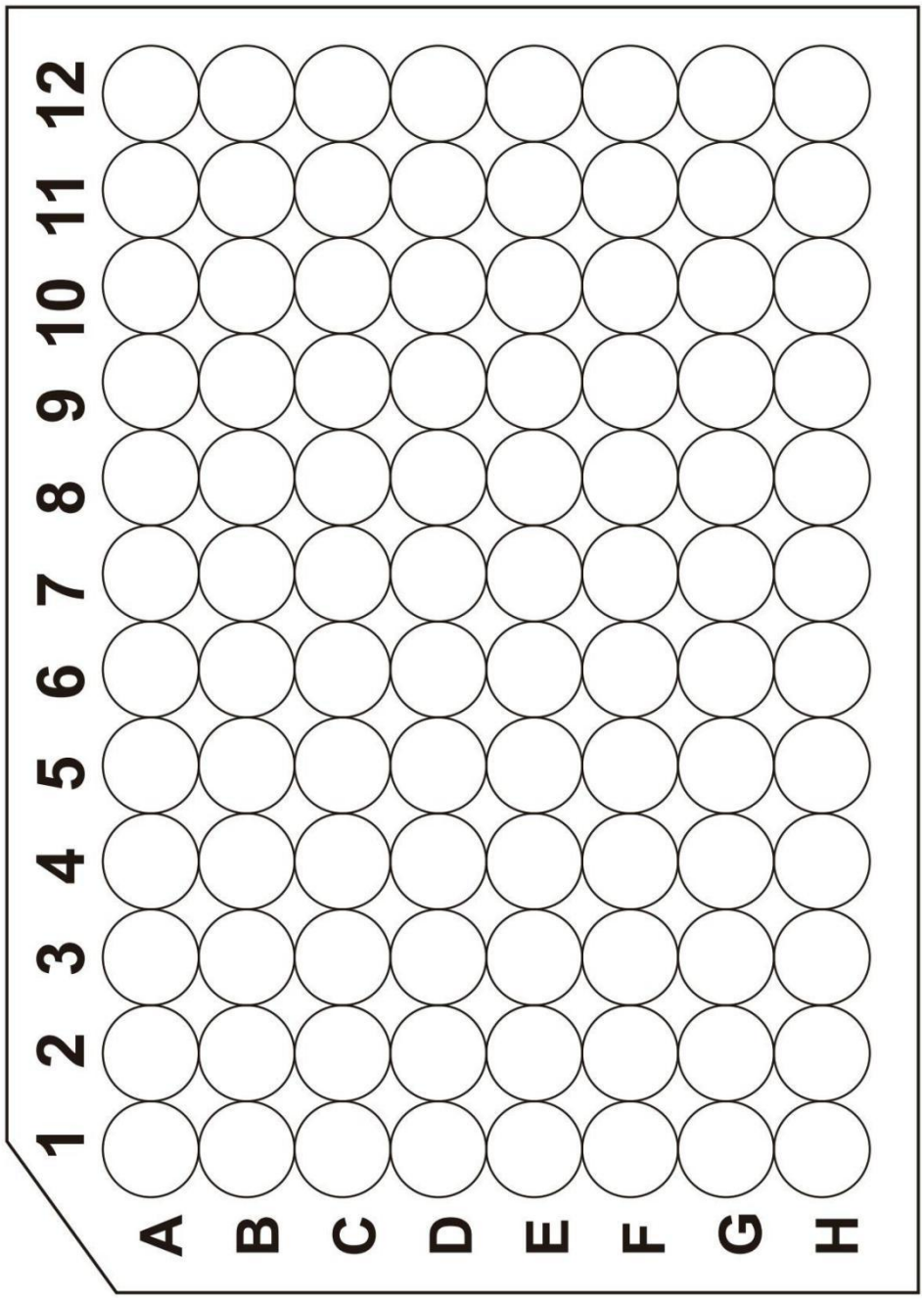
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人/小鼠/大鼠 Activin A Valukine™ ELISA 试剂盒

目录号：VAL203

适用于定量检测天然和重组人/小鼠/大鼠 Activin A 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

info.cn@bio-techne.com

有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202411.1

目录

I. 背景

激活素（Activin）和抑制素（Inhibin）是半胱氨酸结细胞因子 TGF-β超家族的成员，最初是从性腺液中纯化出来的蛋白质，可分别刺激或抑制垂体释放促卵泡激（follicle stimulating hormone,FSH）。后来的研究表明，它们参与了广泛的生物过程，包括组织形态发生和修复、纤维化、炎症、神经发育、造血、生殖系统功能和癌变（1-7）。

Activin 和 Inhibin 是作为前体蛋白产生的。它们的氨基端前肽会被蛋白水解，并促进生物活性蛋白二硫键二聚体的形成（8-10）。Activins 是由各种β亚基（哺乳动物中为βA、βB、βC 和βE）组成的非糖基化同源二聚体或异源二聚体，而 Inhibin 则是由一个独特的α亚基和其中一个β亚基组成的异源二聚体。Activins A 是由两条βA 链组成的同源二聚体。βA 亚基还可以与βB 或βC 亚基异源二聚，分别形成 Activin AB 和 Activin AC（11, 12）。14 kDa 的成熟人类βA 链与牛、猫、小鼠、猪和大鼠的βA 具有 100%的氨基酸序列相同性。许多细胞类型都表达βA 链，包括成纤维细胞和角质形成细胞（13），血管平滑肌细胞（14），上皮细胞和内皮细胞（14, 15），肝细胞（16），破骨细胞和软骨细胞（17, 18），单核细胞和巨噬细胞（14, 19），神经元（20），卵巢和睾丸的体细胞（21）以及垂体前叶的促性腺激素（22）。

Activin A 通过与 2 型丝氨酸/苏氨酸 Activin RIIA 激酶结合，然后与 1 型丝氨酸/苏氨酸激酶 Activin RIB/ALK-4 非共价结合来发挥其生物活性（7, 23, 24）。通过这种受体复合物发出的信号会导致 Smad 激活并调节 activin-responsive 基因的转录（7, 24）。Activin A 的生物活性受多种机制调控（24）。BAMBI、TGF-β RIII 和 Cripto 是细胞相关的分子，它们可作为诱饵受体或限制 Activin A 诱导受体复合物组装的能力（25-27）。通过将βA 亚基掺入 Activin AC 或 Inhibin A 中，可以来阻止细胞内形成 Activin A（3, 11, 12）。由于 Activin A 与α2-巨球蛋白、Follistatin 和 FLRG 结合成非活性复合物，其生物利用率受到限制（28-30）。

II. 概述

A. 检测原理

该测定采用3步定量夹心酶联免疫测定技术。抗人/小鼠/大鼠Activin A特异性捕获抗体被生物素化并结合到链霉亲和素包被的板上。洗板后将检测液、标准品和样品加入孔中，任何存在的人/小鼠/大鼠Activin A都与固定的抗体结合。洗去未结合物质后，在孔中加入抗人/小鼠/大鼠βA亚基的特异性辣根过氧化物酶结合物。洗涤去除未结合的酶结合物后，在孔中加入TMB底物（显色剂），颜色与人/小鼠/大鼠Activin A结合的量成比例；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，人/小鼠/大鼠血清样本、人/小鼠/大鼠血浆样本和人/小鼠/大鼠唾液样本；
- 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RD5-54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，在不同板间分别检测40次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	101	295	498	106	308	529
标准差	4.2	12.5	21.9	8.4	15.5	24.8
CV%	4.2	4.2	4.4	7.9	5.0	4.7

B. 回收率

在测量范围内，对人类样本中掺入三种不同水平的人/小鼠/大鼠Activin A，测定其回收率。

样本类型	平均回收率 (%)	范围 (%)
细胞培养基 (n=4)	105	93-114
人血清样本* (n=4)	109	100-115
人 EDTA 血浆样本* (n=4)	110	95-115
人肝素血浆样本* (n=4)	107	99-113

*在分析之前稀释样品。

C. 灵敏度

对40种检测方法进行了评估，人/小鼠/大鼠Activin A的最小可检测量（MDD）范围为0.75-7.85 pg/mL。平均MDD为3.67 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

该免疫测定法以R&D Systems生产的高纯度CHO细胞表达的重组人/小鼠/大鼠Activin A蛋白校正。

NIBSC/WHO Activin A 第一代参考试剂（91/626），作为效价标准品，在该试剂盒中进行了评估。该标准的剂量反应曲线与Valukine Activin A标准曲线平行。要将Valukine人/小鼠/大鼠Activin A分析获得的样品值转换为近似NIBSC 91/626标称值，请使用以下公式。

NIBSC (91/626)近似值 (mU/mL) = 1.185 × Activin A Valukine检测值 (pg/mL)

E. 线性

为了评估测定的线性，使用含有或掺入高浓度的人/小鼠/大鼠Activin A的人样本，并用标准品稀释液RD5-54稀释，以产生在测定动态范围内的样本值。

稀释倍数		细胞培养基* (n=4)	人血清* (n=4)	人EDTA血浆* (n=4)	人肝素血浆* (n=4)	人唾液* (n=4)
1:2	平均值/期待值 (%)	99	107	102	109	103
	范围 (%)	93-106	105-111	101-104	104-112	99-109
1:4	平均值/期待值 (%)	97	108	104	112	91
	范围 (%)	90-103	101-115	100-111	109-114	82-98
1:8	平均值/期待值 (%)	96	110	107	112	93
	范围 (%)	89-108	107-119	102-116	103-118	____
1:16	平均值/期待值 (%)	102	101	98	105	____
	范围 (%)	96-108	94-116	88-114	88-120	____

*在分析之前稀释样品。

F. 样本预值

人/小鼠/大鼠血清/血浆/唾液 - 本实验评估了人/小鼠/大鼠Activin A的存在。本研究中使用的捐献者没有可用的病史。

人样本	平均值 (pg/mL)	范围 (pg/mL)	标准偏差 (pg/mL)
人血清 (n=35)	352	142-753	129
人EDTA血浆 (n=35)	319	115-665	124
人肝素血浆 (n=35)	316	111-695	126

人样本	平均可检测值 (pg/mL)	%可检测率	范围 (pg/mL)
人唾液 (n=13)	192	85	ND-428

ND=未检出

小鼠样本	平均值 (pg/mL)	范围 (pg/mL)	标准偏差 (pg/mL)
小鼠血清 (n=20)	197	78.1-352	93.1
小鼠EDTA血浆 (n=20)	254	136-471	84.2
小鼠肝素血浆 (n=20)	188	130-272	46.0

大鼠样本	平均值 (pg/mL)	范围 (pg/mL)	标准偏差 (pg/mL)
大鼠血清 (n=8)	306	109-1466	469
大鼠EDTA血浆 (n=9)	214	97.7-312	63.9
大鼠肝素血浆 (n=7)	276	210-342	42.9

细胞上清液：

注：*细胞培养上清液未校正胎牛血清的存在。*

将IMR-90人胎肺成纤维细胞在含有10%胎牛血清的MEM中培养。取样测定人/小鼠/大鼠Activin A，测量值为5608 pg/mL。

将WS-1人胎儿皮肤成纤维细胞在含有10%胎牛血清、NEAA和2 mM L-谷氨酰胺的MEM中培养。然后分别用100 ng/mL LPS、10 ng/mL重组人IL-1β或10 ng/mL重组人TNF-α刺激细胞再刺激24小时。取样并测定人/小鼠/大鼠Activin A的水平。

刺激物	**1天 (pg/mL)**
LPS	8489
IL-1β	21,033
TNF-α	21,098

将CMT-93小鼠直肠癌细胞在含有10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素的MEM中培养。取样测定人/小鼠/大鼠Activin A，测量值为655 pg/mL。

将ST-2小鼠骨髓基质细胞在含有10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素的DMEM中培养。取样测定人/小鼠/大鼠Activin A，测量值为3156 pg/mL。

将LL/2小鼠Lewis肺癌细胞在含有10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素的DMEM中培养。取样测定人/小鼠/大鼠Activin A，测量值为44.3 pg/mL。

将C6大鼠神经胶质瘤细胞在含有10%胎牛血清和2 mM L-谷氨酰胺的DMEM中培养直至汇合。取样测定人/小鼠/大鼠Activin A，测量值为1559 pg/mL。

将C58（NT）D大鼠胸腺淋巴瘤细胞在含有10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和4.5 g/L葡萄糖的DMEM中培养。取样测定人/小鼠/大鼠Activin A，测量值为21.0 pg/mL。

G. 特异性

此ELISA法可检测天然及重组人/小鼠/大鼠Activin A。

将以下蛋白用标准品稀释液RD5-54配制成50 ng/mL的浓度来检测交叉反应。将50 ng/mL的干扰蛋白掺入中值人/小鼠/大鼠Activin A对照品中，来检测对其干扰。没有观察到明显的交叉反应或干扰。

Recombinant human:		**Recombinant mouse:**	**Recombinant rat:**
Activin AC Heterodimer	BMP-15	Activin C	Agrin
Activin B	Cripto	Activin RIB	ALK-7
Activin C	DAN	Activin RIIB	MIS
Activin RIA	Endoglin	ALK-1	**Other recombinants:**
Activin RIB	Follistatin 288	BAMBI	zebrafish BMP-2
Activin RIIA	Follistatin 300	BMPRIA	porcine TGF-β2
Activin RIIB	Follistatin 315	BMPRIB	amphibian TGF-β5
ALK-1	Inhibin B	BMP-3b/GDF-10	**Natural proteins:**
BAMBI	Lefty A	Cripto	porcine TGF-β1
BMPRII	MIS	DAN	
BMP-1/PCP	Osteoactivin	Endoglin	
BMP-2	LAP	Lefty	
BMP-3	TGF-α	MIS	
BMP-3b/GDF-10	TGF-β1	Noggin	
BMP-4	TGF-β1.2	Osteoactivin	
BMP-5	TGF-β2		
BMP-6	TGF-β3		
BMP-8b	TGF-βRII		
BMP-10	TGF-βRIII/betaglycan		

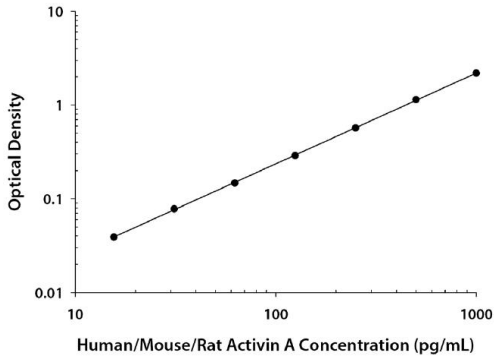
在该测定中，重组人Inhibin A交叉反应率约为0.2%。

在该检测中，重组人Activin AB交叉反应率约为0.45%。

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。



(pg/mL)	O.D.	Average	Corrected
0	0.051 0.051	0.051	—
15.6	0.089 0.090	0.090	0.039
31.3	0.127 0.130	0.129	0.078
62.5	0.196 0.199	0.198	0.147
125	0.330 0.350	0.340	0.289
250	0.601 0.639	0.620	0.569
500	1.180 1.199	1.190	1.139
1000	2.207 2.258	2.233	2.182

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Streptavidin Microplate	包被链霉亲和素的96孔聚苯乙烯板，8孔×12条	1块板
Activin A Biotinylated Antibody	抗人/小鼠/大鼠Activin A生物素标记抗体	1瓶
Activin A Conjugate	酶标检测抗人/小鼠/大鼠Activin A抗体	1瓶
Activin A Standard	重组人Activin A标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1-98	蓝色检测液	1瓶
Calibrator Diluent RD5-54	标准品稀释液用于稀释标准品和样品	1瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	2瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	8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="9">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="8">2-8°C储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>Activin A生物素标记抗体</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>检测液RD1-98</td></tr>
<tr><td>标准品稀释液RD5-54</td></tr>
<tr><td>标准品</td></tr>
<tr><td>TMB底物溶液</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 °C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
- 1000 mL量筒
- 水平振荡器（0.12" 轨道），转速：500±50 rpm
- 标准品稀释用聚丙烯试管

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

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清液 - 通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤ -20°C的温度下，避免反复冻融。样品可能需要用标准品稀释液RD5-54稀释。

注：*含有生物素的培养基，例如RPMI和McCoy's培养基，不适合用于本测定。用于制备细胞培养基的动物血清可能含有内源性Activin A。为了获得最佳结果，在测定Activin A 时，请勿使用动物血清来培养细胞。如果动物血清用作培养基的补充剂，则应采取预防措施，并制备相应的对照品，以确定Activin A的基线浓度。*

人血清 - 使用血清分离管 (SST)，将样本在室温下凝固 30分钟，然后在1000 × g的离心力下离心15分钟。分离血清并立即进行检测，或分装并储存在≤ -20 °C。避免反复冻融循环。样本可能需要用标准品稀释液RD5-54进行稀释。

小鼠血清 - 将血液样本在室温下凝固2小时，然后在2000 × g离心20分钟。分离血清并立即进行检测，或分装并储存在≤-20 °C。避免反复冻融循环。样品可能需要用标准品稀释液RD5-54稀释。

大鼠血清 - 将血液样本在室温下凝固2小时，然后在1000 × g离心30分钟。分离血清并立即进行检测，或分装并储存在≤ -20 °C。避免反复冻融循环。样品可能需要用标准品稀释液RD5-54稀释。

血浆 - 使用EDTA或肝素作为抗凝剂收集血浆。然后以1000 × g离心15分钟。需在30分钟内收集血浆样本之后即刻用于检测，或者分装，≤ -20°C贮存备用。立即检测或分装并储存在 ≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液RD5-54稀释。

注：*柠檬酸盐血浆在本检测中未经验证。*

严重溶血样本不适合用于此检测。

唾液 - 使用收集装置（例如Salivette或等效装置）收集唾液。立即检测或等分样品并将其储存在≤ -20 °C的温度下。避免反复冻融循环。样品可能需要用标准品稀释液RD5-54稀释。

注：*唾液收集器不得具有任何蛋白质结合或过滤功能。*

B. 样品准备

人/小鼠/大鼠血清、血浆和唾液样本建议至少进行 2 倍稀释。建议的 2 倍稀释方法为：150 µL 样本+150 µL 标准品稀释液 RD5-54。最佳稀释倍数应由用户自行确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

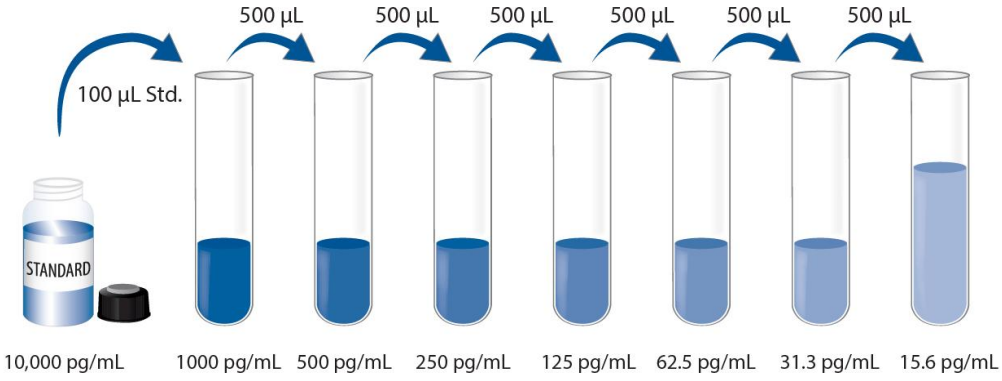
注：*在人/小鼠/大鼠唾液中发现了高浓度的人/小鼠/大鼠Activin A。建议使用口罩和手套以保护试剂盒免受污染。*

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。将40 mL洗涤缓冲液浓缩液加入960 mL去离子水或蒸馏水中，制备1000 mL洗涤缓冲液。

Activin A 标准品：复溶体积请参考瓶身标签*，用去离子水或蒸馏水复溶Activin A标准品，得到浓度为10000 pg/mL标准品储备母液。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。

将900 µL标准品稀释液RD5-54移入1000 pg/mL的管中。剩余的管中加入500 µL标准品稀释液RD5-54。使用标准品储备母液制作稀释系列（如下图）。在进行下一次转移前，充分混匀每支管。1000 pg/mL的标准品作为最高标准品。标准品稀释液RD5-54作为零标准品（0 pg/mL）。



D. 微孔板制备

1. 从板架上取下多余的微孔板条，放回装有干燥剂包的铝箔袋中，并重新封好。

2. 将200 µL的抗Activin A生物素标记抗体加入所有孔中。盖好，在水平振荡器（0.12" 轨道）上以500±50 rpm的转速室温孵育15分钟。

3. 吸取每个孔中的液体并清洗，重复此过程两次。使用洗瓶、多通道洗板器或自动洗板机洗板，用洗涤缓冲液（400 µL）填充每个孔进行清洗。每个步骤都必须彻底去除液体，以确保良好的检测结果。最后一次清洗后，通过吸取或倾倒去除剩余的洗涤缓冲液。将板倒置并用干净的纸巾吸干。

4. 洗涤后立即进行测定。切勿让孔干燥。

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；
- 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；
- 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，将所有其他试剂和样品带至室温。建议对所有标准品和样品进行复孔检测。

注：*在人/小鼠/大鼠唾液中发现了高浓度的人/小鼠/大鼠Activin A。建议使用口罩和手套以保护试剂盒免受污染。*

1. 按照上一节的说明，准备好所有需要的试剂，标准品、微孔板和样本；
2. 向每个孔中加入100 µL检测液RD1-98。
3. 分别将不同浓度标准品和实验样本加入相应孔中，每孔100 µL。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；
4. 用封板膜封住微孔板。**用水平微孔板振荡器（0.12" 轨道），转速：500±50 rpm室温孵育3小时。**
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5次，共洗6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200 µL Activin A酶标检测抗体。用新的封板膜封住反应孔，**用水平微孔板振荡器室温孵育1小时**；
7. 重复第5步洗板操作；
8. 在每个微孔内加入200 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50 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log/log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log/log图上的点绘制最佳拟合曲线。数据可以通过绘制人/小鼠/大鼠Activin A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。

    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