PRODUCT INFORMATION & MANUAL

Mouse/Rat PDGF-AB Valukine™ ELISA

Catalog Number: VAL629

For the quantitative determination of natural and recombinant mouse/rat PDGF-AB 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 202308.1

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I. BACKGROUND

The platelet-derived growth factor (PDGF) family consists of proteins derived from four genes (PDGF-A, -B, -C and -D) that form four disulfide-linked homodimers (PDGF-AA, -BB, -CC, and -DD) and one heterodimer (PDGF-AB) (1-3). These proteins and the related VEGF family proteins share the conserved PDGF/VEGF homology domain characterized by a pattern of highly conserved cysteine residues, which form the cystine knot motif. PDGF-A is synthesized as a prepro-protein with a signal peptide and an N-terminal pro-peptide (4, 5). Two PDGF-A splice variants, with or without the short stretch of conserved basic C-terminal residues, which comprises the cell retention motif, are expressed (6). The cell retention motif interacts with negatively charged glycosaminoglycans inside the cell and in the extracellular matrix. PDGF-B is also synthesized as a pre-pro-protein, which has a signal peptide, an N-terminal pro-peptide and a C-terminal extension that also contains the conserved cell retention motif (5-7). In cells that express both the -A and -B chains, the individual chains are assembled stochastically into disulfide-linked inactive homo- or hetero-dimeric precursors in the endoplasmic reticulum (1-3). Within the trans-Golgi network, these precursors then undergo intracellular proteolytic processing necessary for the secretion of the biologically active mature proteins. Secreted PDGF dimers containing the retention motif are matrix-bound and need to undergo additional proteolytic processing before they can be released from the extracellular matrix. PDGF-A and -B isoforms were originally isolated from platelets but were subsequently found to be produced by multiple cell types including megakaryocytes, fibroblasts, keratinocytes, vascular smooth muscle cells, endothelial cells, neurons, Schwann cells, and macrophages (3). The amino acid sequences of mature mouse and rat PDGF-A (short isoform) are 99% identical (4, 5). Mature mouse and rat PDGF-B also share 99% amino acid sequence identity (5, 7).

PDGF family proteins regulate diverse cellular functions by binding to and inducing the homo- or hetero-dimerization of two receptor subunits (PDGF Rα and Rβ) (1-3). Both subunits belong to the class III subfamily of receptor tyrosine kinases. PDGF-AB can induce α/α homodimerization as well as α/β hetero-dimerization. Ligand-induced receptor dimerization results in autophosphorylation in trans, resulting in the activation of several intracellular signaling pathways that have important roles in the regulation of cell growth and differentiation, as well as in wound healing.

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse/rat PDGF-AB has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse/rat PDGF-AB present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for mouse/rat PDGF-AB is pipetted into the wells. After washing away any unbound substances, Streptavidin-HRP is pipetted into the wells. Following a wash to remove any unbound reagent, TMB substrate solution (Chromogenic agent) is added to the wells and color develops in proportion to the amount of mouse/rat PDGF-AB 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 supernates and mouse/rat serum.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent (1×) 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.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	257.5	65.8	17.3	256.7	66.4	16.7
Standard Deviation	5.1	1.0	0.5	5.5	1.3	1.1
CV%	2.0	1.5	2.8	2.1	2.0	6.8

B. RECOVERY

The recovery of mouse/rat PDGF-AB spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 103.4 to 111.5% with an average of 107.3%.

The recovery of mouse/rat PDGF-AB spiked to different levels throughout the range of the assay in mouse/rat serum was evaluated. The recovery ranged from 98.3 to 124.3% with an average of 108.4%

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse/rat PDGF-AB is typically less than 1.52 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 *E. coli*-expressed recombinant rat PDGF-AB produced at R&D Systems.

E. LINEARITY

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

Dilution	Average % of Expected	Range (%)
1:2	95.3	92.4-100.8
1:4	93.2	88.6-98.7
1:8	91.3	85.8-99.2
1:16	87.3	78.1-102.7

F. SAMPLE VALUES

Serum - Three mouse serum samples were evaluated for the presence of PDGF-AB in this assay. All samples measured ranged from 1488 to 3630 pg/mL with an average of 2824 pg/mL. Three rat serum samples were evaluated for the presence of PDGF-AB in this assay. All samples measured ranged from 275.6 to 317.6 pg/mL with an average of 302.0 pg/mL.

G. SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant mouse:	**Other recombinants:**	**Natural proteins:**
PDGF-CC	equine PDGF-BB	porcine PDGF
	human PDGF-BB	
Recombinant rat:		
PDGF-AA		
PDGF-BB		

Recombinant mouse (rm) PDGF-Rα/Fc Chimera and rmPDGF-Rβ/Fc Chimera do not cross-react in this assay but do interfere at concentrations > 781 pg/mL and 1563 pg/mL respectively.

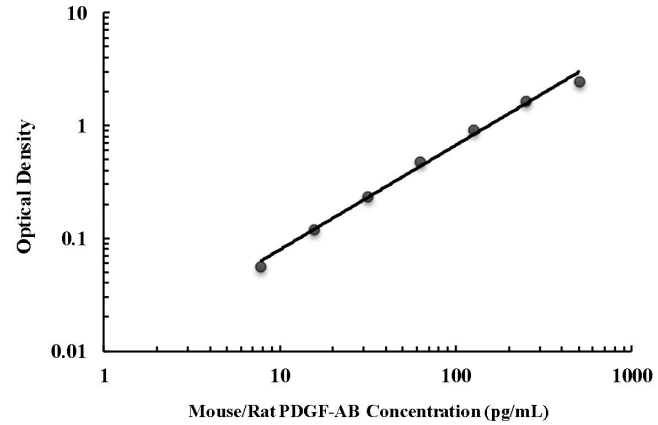
The following factors were assayed and exhibited the following cross-reactivities:

Factor	Concentration Tested (pg/mL)	Observed Value (pg/mL)	% Cross-reactivity
hPDGF	625	254	40.6
rhPDGF-AA	50,000	71	0.14
rhPDGF-AB	625	418	66.9

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	OD	Average	Corrected
0	0.077 0.081	0.079	-
7.8	0.136 0.134	0.135	0.056
15.6	0.199 0.198	0.199	0.120
31.3	0.310 0.318	0.314	0.235
62.5	0.558 0.562	0.560	0.481
125	0.993 1.025	1.009	0.930
250	1.741 1.744	1.743	1.664
500	2.543 2.569	2.556	2.477

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse/Rat PDGF-AB Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse antibody against mouse/rat PDGF-AB.	1 plate
Mouse/Rat PDGF-AB Standard	Recombinant rat PDGF-AB in a buffered base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Mouse/Rat PDGF-AB Detection Antibody	Biotinylated mouse/rat PDGF-AB antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent Concentrate (2×)	A 2× concentrated buffered protein base used to dilute standard and samples.	1 vial
Detection Antibody Diluent Concentrate (10×)	A 10× concentrated buffered diluent used to dilute detection antibody.	1 vial
Reagent Diluent Concentrate (10×)	A 10× concentrated buffered protein base used to dilute HRP.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution.	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strip.	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>Streptavidin-HRP A</td><td rowspan="4">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.
Standards may be stored for up to 1 month at -20 °C.*</td></tr>
<tr><td>Detection Antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Calibrator Diluent Concentrate (2×)</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>Detection Antibody Diluent Concentrate (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Detection Antibody Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Reagent Diluent Concentrate (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent 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.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500±50 rpm.

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 protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

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

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

B. SAMPLE PREPARATION

Cell culture supernate samples recommend a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

Mouse serum samples recommend a 30-fold dilution. A suggested 30-fold dilution is 5 µL of sample + 145 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

Rat serum samples recommend a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

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

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

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

Detection Antibody Diluent (1×) - Use deionized or distilled water to prepare Detection Antibody Diluent (1×)

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

Detection Antibody (1×)- Centrifuge briefly before opening. Reconstitution volume refer to vial label to prepare Detection Antibody (100×). Allow the Detection Antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Aliquot and store if needed. Dilute to Detection Antibody (1×) with Detection

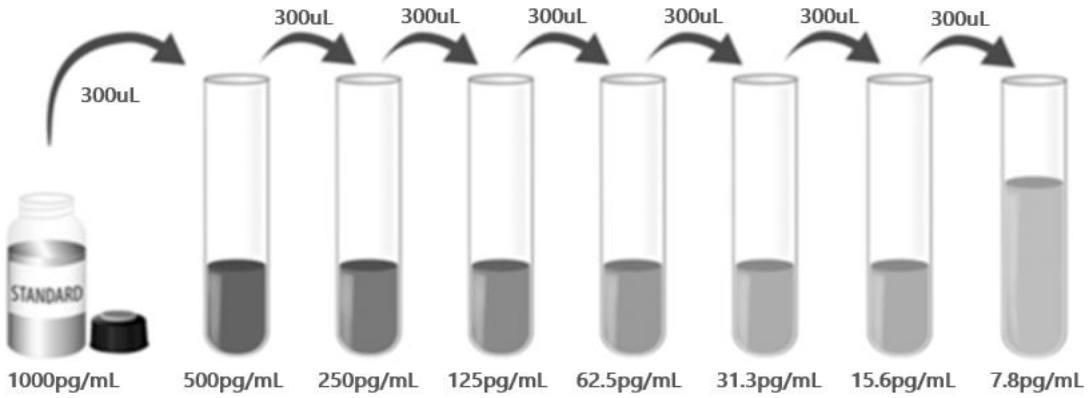
Antibody Diluent (1×). Prepare at least 15 minutes prior to use.

Streptavidin-HRP A (1×) - Centrifuge briefly before opening. Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Mouse/Rat PDGF-AB Standard - Centrifuge briefly before opening. Refer to the vial label for the reconstitution volume*. This reconstitution produces a stock solution of 1000 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 300 µL of the appropriate Calibrator Diluent (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. The Calibrator Diluent (1×) serves as the zero standard (0 pg/mL).



D. 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

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 100 µL of standard, prepared sample or control per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500±50 rpm**. A plate layout is provided for a record of standards and samples assayed. (Samples may require dilution. See Sample Preparation section.)
4. 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.
5. Add 100 µL of the Detection Antibody (1×) diluted in Detection Antibody Diluent, to each well. Cover with a new adhesive strip and **incubate 2 hours at room temperature on a horizontal orbital microplate shaker set at 500±50 rpm**.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP A to each well. Cover the plate and **incubate for 30 minutes at room temperature. Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light.**
10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. 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.

12. 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 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 mouse/rat PDGF-AB 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.

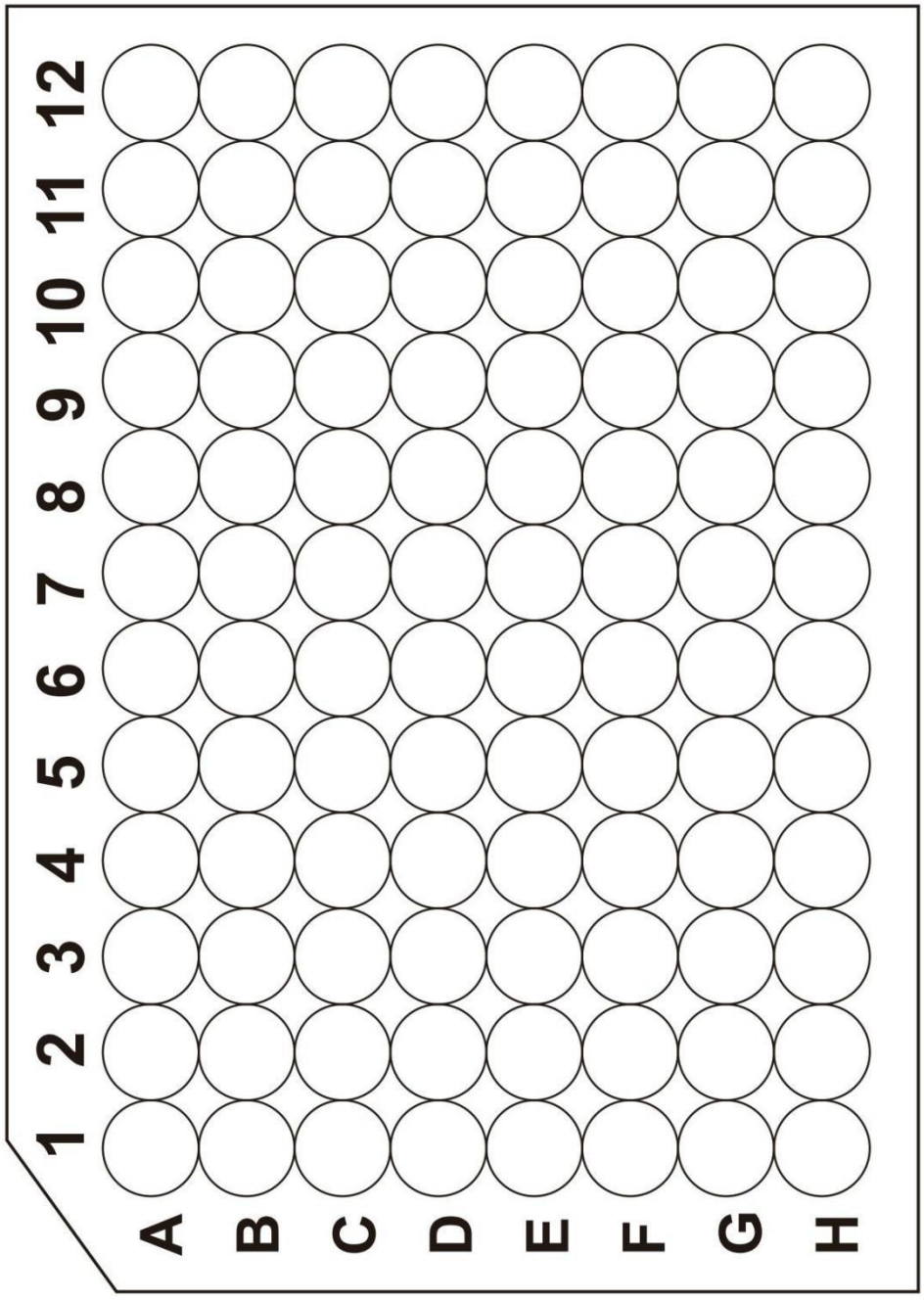
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Fredriksson, L. et al. (2004) Cytokine and Growth Factor Review 15:197.
2. Li, X. and U. Eriksson (2003) Cytokine Growth Factor Rev. 14:91.
3. Heldin, C-H. and B. Westermark (1999) Physiol. Rev. 79:1283.
4. Rorsman, F. and C. Betsholtz (1992) Growth Factors 6:303.
5. Herren, B. et al. (1993) Biochim. Biophys. Acta 1173:294.
6. Ostman, A. et al. (1991) Cell Regul. 2:503.
7. Bonthron, D.T. et al. (1991) Genomics 10:287.

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 PDGF-AB Valukine™ ELISA 试剂盒

目录号：VAL629

适用于定量检测天然和重组小鼠/大鼠 PDGF-AB 的浓度

科研专用，不可用于临床诊断

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版本号 202308.1

目录

I. 背景

血小板衍生生长因子（PDGF）家族由衍生自四个基因的蛋白质（PDGF-A、-B、-C和-D）组成，这些蛋白质可形成四种二硫键连接的同源二聚体（PDGF-AA、-BB、-CC和-DD）和一种异源二聚体（PDGF-AB）（1-3）。这些蛋白和相关的VEGF家族蛋白共享保守的PDGF/VEGF同源结构域，其特征是形成胱氨酸结基序的高度保守的半胱氨酸残基模式。PDGF-A被合成为具有信号肽和N-末端前肽的前体蛋白（4，5）。表达了两种PDGF-A剪接变体，具有或不具有短链的保守的碱性C末端残基，其包括细胞保留基序（6）。细胞保留基序与细胞内和细胞外基质中带负电荷的糖胺聚糖相互作用。PDGF-B也被合成为前原蛋白，其具有信号肽、N末端原肽和C末端延伸，C末端延伸也包含保守的细胞保留基序（5-7）。在同时表达-A和-B链的细胞中，单个链在内质网中随机组装成二硫键连接的无活性同源或异源二聚体前体（1-3）。在反式高尔基体网络中，这些前体随后经历分泌生物活性成熟蛋白所必需的细胞内蛋白水解处理。分泌的含有保留基序的PDGF二聚体与基质结合，需要经过额外的蛋白水解处理才能从细胞外基质中释放出来。PDGF-A和-B亚型最初是从血小板中分离出来的，但后来发现由多种细胞类型产生，包括巨核细胞、成纤维细胞、角质形成细胞、血管平滑肌细胞、内皮细胞、神经元、雪旺细胞和巨噬细胞（3）。成熟小鼠和大鼠PDGF-A（短亚型）的氨基酸序列99%相同（4，5）。成熟小鼠和大鼠PDGF-B也共享99%的氨基酸序列同一性（5，7）。

PDGF家族蛋白通过结合并诱导两个受体亚基（PDGF Rα和Rβ）的同源或异源二聚来调节不同的细胞功能（1-3）。这两个亚基都属于受体酪氨酸激酶的III类亚家族。PDGF-AB可诱导α/α同源二聚以及α/β异源二聚。配体诱导的受体二聚化导致反式中的自身磷酸化，导致几种细胞内信号通路的激活，这些信号通路在调节细胞生长和分化以及伤口愈合中具有重要作用。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠/大鼠PDGF-AB抗体包被于微孔板上，样品和标准品中的小鼠/大鼠PDGF-AB会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗小鼠/大鼠PDGF-AB检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶（Streptavidin-HRP）孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合的目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

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

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，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。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	257.5	65.8	17.3	256.7	66.4	16.7
标准差	5.1	1.0	0.5	5.5	1.3	1.1
CV%	2.0	1.5	2.8	2.1	2.0	6.8

B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的小鼠/大鼠PDGF-AB，测定其回收率。回收率范围在103.4-111.5%，平均回收率在107.3%。

在小鼠/大鼠血清样本中掺入检测范围内不同水平的小鼠/大鼠PDGF-AB，测定其回收率。回收率范围在98.3-124.3%，平均回收率在108.4%。

C. 灵敏度

小鼠/大鼠PDGF-AB的最低可测剂量（MDD）一般小于1.52 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*E. coli*表达的高纯度重组大鼠PDGF-AB蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠/大鼠PDGF-AB，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。

稀释倍数	平均值 (%)	范围 (%)
1:2	95.3	92.4-100.8
1:4	93.2	88.6-98.7
1:8	91.3	85.8-99.2
1:16	87.3	78.1-102.7

F. 样本预值

血清样本 - 使用本试剂盒检测了3份小鼠血清样本中PDGF-AB的水平。3份样本的检测值范围为1488-3630 pg /mL，平均值为2824 pg/mL。使用本试剂盒检测了3份大鼠血清样本中PDGF-AB的水平。3份样本的检测值范围为275.6-317.6 pg /mL，平均值为302.0 pg/mL。

G. 特异性

将以下因子配制成50 ng/mL的浓度来检测没有观察到明显的交叉反应。

Recombinant mouse:	**Other recombinants:**	**Natural proteins:**
PDGF-CC	equine PDGF-BB	porcine PDGF
	human PDGF-BB	
Recombinant rat:		
PDGF-AA		
PDGF-BB		

重组小鼠（rm）PDGF-Rα/Fc嵌合体和rmPDGF-Rβ/Fc嵌合体在该测定中不发生交叉反应，但在浓度分别大于781 pg/mL和1563 pg/mL时会产生干扰。

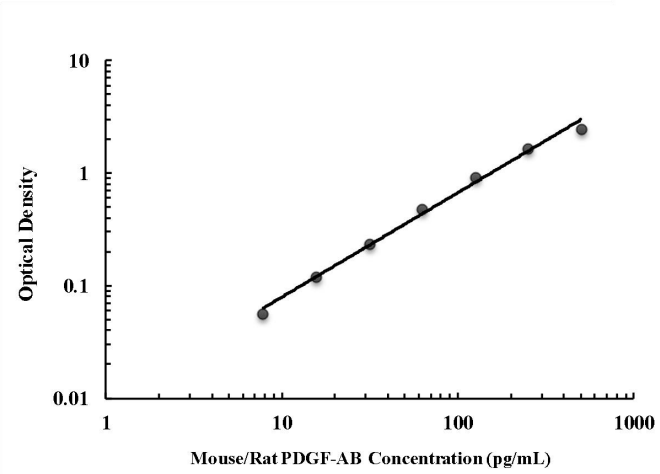
对以下因子进行检测，观察到以下交叉反应：

Factor	Concentration Tested (pg/mL)	Observed Value (pg/mL)	% Cross-reactivity
hPDGF	625	254	40.6
rhPDGF-AA	50,000	71	0.14
rhPDGF-AB	625	418	66.9

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	OD	Average	Corrected
0	0.077 0.081	0.079	-
7.8	0.136 0.134	0.135	0.056
15.6	0.199 0.198	0.199	0.120
31.3	0.310 0.318	0.314	0.235
62.5	0.558 0.562	0.560	0.481
125	0.993 1.025	1.009	0.930
250	1.741 1.744	1.743	1.664
500	2.543 2.569	2.556	2.477

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse/Rat PDGF-AB Microplate	包被小鼠抗小鼠/大鼠 PDGF-AB 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse/Rat PDGF-AB Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Mouse/Rat PDGF-AB Detection Antibody	生物素化的小鼠/大鼠 PDGF-AB 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Calibrator Diluent Concentrate (2×)	浓缩的标准品稀释液（2×）用于稀释标准品和样本。	1 瓶
Detection Antibody Diluent Concentrate (10×)	浓缩的检测抗体稀释液（10×）用于稀释检测抗体	1 瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液（10×）用于稀释 HRP	1 瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的 HRP	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
TMB Substrate	TMB ELISA 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="9">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP A</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*
标准品-20℃储存，最多 30 天*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</td></tr>
<tr><td>标准品稀释液（2×）</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>检测抗体稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×检测抗体稀释液，多余的丢弃</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液，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
- 水平轨道式微孔板振荡器（500±50 rpm）

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。使用后请彻底洗手。

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。样本需要用标准品稀释液（1×）稀释。

血清样本: 用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后1000 × 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。样 本需要用标准品稀释液（1×）稀释。

B. 样本准备工作

细胞上清样本建议用标准品稀释液（1×）2倍稀释后进行检测，例如：100 µL样本+100 µL标准品稀释液（1×）。最佳稀释度应由最终用户确定。

小鼠血清样本建议用标准品稀释液（1×）30倍稀释后进行检测，例如：5 µL样本+145 µL标准品稀释液（1×）。最佳稀释度应由最终用户确定。

大鼠血清样本建议用标准品稀释液（1×）4倍稀释后进行检测，例如：50 µL样本+150 µL标准品稀释液（1×）。最佳稀释度应由最终用户确定。

C. 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浓缩洗涤液用蒸馏水或去离子水稀释配制成500mL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×）。

检测抗体稀释液（1×）：使用去离子水或蒸馏水稀释配制成检测抗体稀释液（1×）。

试剂稀释液（1×）：使用去离子水或蒸馏水稀释配制成试剂稀释液（1×）。

检测抗体（1×）：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震摇至少15分钟，使其充分溶解。如有需要分装保存。再用检测抗体稀释液（1×）稀释至检测抗体（1×），至少在使用前15分钟准备。

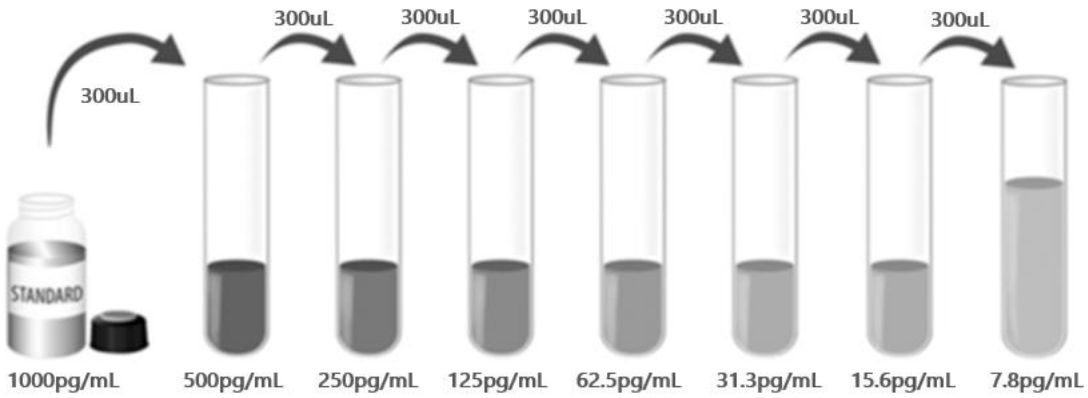
链霉亲和素- HRP A（1×）：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP A（200×）稀释至工作浓度。

小鼠/大鼠PDGF-AB标准品：开盖前请瞬时离心。冻干标准品的重溶体积请参考瓶身标签，

得到浓度为1000 pg/mL标准品母液。轻轻震摇至少15分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

向各稀释管中加入300 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。500 pg/mL管作标准曲线最高点，标准品稀释液（1×）可用作标准品零点（0 pg/mL）。



D. 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. 分别将不同浓度标准品、实验样本或质控品加入相应孔中，每孔100 µL。用封板膜封住反应孔，**室温500±50 rpm水平振荡孵育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；（样本需要稀释，详情参见样本制备部分。）
4. 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；
5. 在每个微孔内加入100 µL配制好的检测抗体（1×）。用封板膜封住反应孔，**室温500±50 rpm水平振荡孵育2小时；**
6. 重复第4步洗板操作；
7. 在每个微孔内加入100 µL稀释好的链霉亲和素- HRP A 工作液。用封板膜封住反应孔，**静置室温孵育30分钟，注意避光；**
8. 重复第4步洗板操作；
9. 在每个微孔内加入100 µL TMB底物溶液，**静置室温孵育30分钟，注意避光；**
10. 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；
11. 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；
12. **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。数据可以通过绘制小鼠/大鼠PDGF-AB浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

    如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

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2. Li, X. and U. Eriksson (2003) Cytokine Growth Factor Rev. 14:91.
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4. Rorsman, F. and C. Betsholtz (1992) Growth Factors 6:303.
5. Herren, B. et al. (1993) Biochim. Biophys. Acta 1173:294.
6. Ostman, A. et al. (1991) Cell Regul. 2:503.
7. Bonthron, D.T. et al. (1991) Genomics 10:287.

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