PRODUCT INFORMATION & MANUAL

Mouse Thrombopoietin Valukine™ ELISA

Catalog Number: VAL653

For the quantitative determination of natural and recombinant mouse Thrombopoietin (Tpo) 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 202506.1

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I. BACKGROUND

Thrombopoietin (Tpo), also known as megakaryocyte growth and development factor (MGDF), the ligand for the receptor encoded by the *c-Mpl* proto-oncogene, is a key regulator of megakaryocytopoiesis and thrombopoiesis *in vitro* and *in vivo* (1). Tpo has been purified and cloned from several species including human, mouse, rat, and canine (2-7). The proteins from the various species are highly conserved, exhibiting from 69-75% sequence identity at the amino acid (aa) sequence level. The mouse Tpo cDNA sequence encodes a 356 aa residue protein with a 21 aa residue signal peptide that is cleaved to yield the 335 aa residue mature protein (3, 5). Mature Tpo can be divided into two domains: the amino-terminal half with homology to erythropoietin (Epo) and the carboxy-terminal half rich in serine, threonine and proline residues and containing seven potential N-linked glycosylation sites. The Epo-domain of mouse Tpo shows approximately 21% aa sequence identity to mouse Epo and has been shown to activate the *c-Mpl* receptor. The carboxy terminus domain of Tpo has been shown to regulate the specific activity and circulating half-life of Tpo. The carboxy-terminal may also have a role in promoting the efficient biosynthesis and secretion of Tpo (8). The cDNA for a variant form of Tpo (Tpo-2) with a four aa residue deletion within the Epo homology domain has been isolated which is very poorly secreted and lacks Tpo activity (9). Tpo mRNA is expressed predominantly in adult and fetal liver, kidney and bone marrow stromal cells (5).

The receptor for Tpo (*c-Mpl*) has been cloned from both human and mouse cells (10, 11). The *c-Mpl* proto-oncogene was originally identified as the cellular homolog of a retroviral oncogene, *v-Mpl* (12). *C-Mpl* is a member of the cytokine receptor superfamily. Two cDNA clones encoding the transmembrane receptor and a soluble form of the receptor have been isolated from mouse cells (11). The mouse *c-Mpl* proto-oncogene has been localized to the C-band of mouse chromosome 4. The *v-Mpl* oncogene of the myeloproliferative leukemia virus (MPLV) encodes a fusion protein containing retroviral env sequences fused to a truncated Tpo receptor (10-12). Tpo receptor expression is restricted to hematopoietic tissues. Cells known to express Mpl include platelets, megakaryocytes and megakaryocyte progenitor cells (13).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse Tpo has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse Tpo present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse Tpo is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of mouse Tpo 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, mouse plasma and mouse 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 RD5-3 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)	123	455	2364	119	457	2389
Standard Deviation	9.8	17.0	58.4	9.6	21.7	115
CV%	8.0	3.7	2.5	8.1	4.7	4.8

B. RECOVERY

The recovery of mouse Tpo spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture media (n=5)	97	87-115
Mouse serum* (n=5)	100	88-111
Mouse EDTA plasma* (n=5)	104	87-116
Mouse citrate plasma* (n=5)	104	88-113

*Samples were diluted prior to assay as directed in the Sample Preparation section.

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse Tpo is typically less than 20 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 NS0-expressed recombinant mouse Tpo produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, five or more samples containing and/or spiked with high concentrations of mouse Tpo in each matrix were diluted with Calibrator Diluent RD5-3 and assayed. Results from typical sample dilutions are shown.

Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	Observed / Expected x 100
Cell culture media	Neat	3060	——	——
	1:2	1462	1530	96%
	1:4	759	765	99%
	1:8	358	382	94%
	1:16	169	191	88%
Mouse serum*	Neat	4029	——	——
	1:2	2139	2014	106%
	1:4	1062	1007	105%
	1:8	542	504	108%
	1:16	275	252	109%
Mouse EDTA plasma*	Neat	3805	——	——
	1:2	2012	1902	106%
	1:4	1005	951	106%
	1:8	510	476	107%
	1:16	272	238	114%
Mouse citrate plasma*	Neat	3368	——	——
	1:2	1789	1684	106%
	1:4	885	842	105%
	1:8	442	421	105%
	1:16	199	210	95%

* Samples were diluted prior to assay as directed in the Sample Preparation section.

F. SAMPLE VALUES

Mouse serum/ plasma - Samples were evaluated for the presence of mouse Tpo in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Mouse serum (n=20)	4627	2972-6645	992
Mouse EDTA plasma (n=20)	1771	724-3706	710
Mouse citrate plasma (n=20)	1754	841-3544	937

G. SPECIFICITY

This assay recognizes natural and recombinant mouse Tpo.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-3 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse Tpo control were assayed for interference. No significant cross-reactivity or interference was observed.

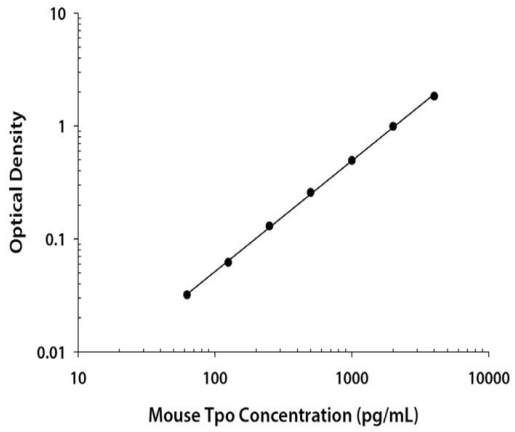
Recombinant mouse:		**Recombinant human:**
C10	IL-10 R	Epo
G-CSF	IL-12	
GM-CSF	IL-13	
IFN-γ	JE/MCP-1	
IL-1α	LIF	
IL-1β	M-CSF	
IL-2	MIP-1α	
IL-3	MIP-1β	
IL-4	MIP-2	
IL-5	SCF	
IL-6	TNF-α	
IL-7	Tpo R	
IL-9	VEGF	
IL-10		

Recombinant human Tpo (NS0-expressed) does not interfere but does cross-react approximately 0.65% 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	0.039 0.041	0.040	—
62.5	0.071 0.073	0.072	0.032
125	0.102 0.102	0.102	0.062
250	0.168 0.172	0.170	0.130
500	0.297 0.298	0.298	0.258
1000	0.533 0.535	0.534	0.494
2000	1.024 1.040	1.032	0.992
4000	1.868 1.879	1.874	1.834

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse Tpo Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse Tpo.	1 plate
Mouse Tpo Conjugate	An antibody specific for mouse Tpo conjugated to horseradish peroxidase.	1 vial
Mouse Tpo Standard	Recombinant mouse Tpo in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Mouse Tpo Control	Recombinant mouse Tpo in a buffered protein base; lyophilized. The assay value of the control should be within the range specified on the vial label.	1 vial
Assay Diluent RD1-23	A buffered protein base.	1 vial
Calibrator Diluent RD5-3	A buffered protein base used to dilute standard and samples.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant.	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution.	2 vials
Stop Solution	Diluted hydrochloric 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>Wash Buffer (1×)</td><td rowspan="5">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Assay Diluent RD1-23</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Calibrator Diluent RD5-3</td><td rowspan="2">Use a new standard and control for each assay. Discard within 8 hours of reconstitution.</td></tr>
<tr><td>Control</td></tr>
<tr><td>Standard</td><td rowspan="2">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>
<tr><td>Microplate Wells</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.
- Test tubes for dilution of standards and samples.

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 RD5-3.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x 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 RD5-3.

Plasma - Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 20 minutes at 2000 x 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 RD5-3.

Note: *Heparin plasma is not recommended for use in this assay*.

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

B. SAMPLE PREPARATION

Mouse serum and plasma samples recommend a 5-fold dilution prior to assay. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD5-3. Mix well and allow samples to equilibrate for 20 minutes prior to assay. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse Tpo Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

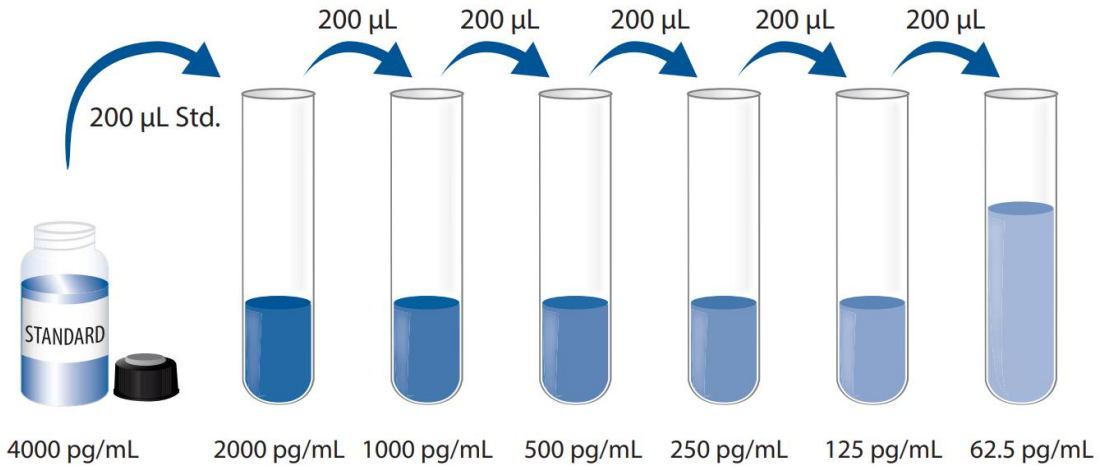
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Mouse Tpo Standard- Refer to the vial label for the reconstitution volume* Reconstitute the Mouse Tpo Standard with Calibrator Diluent RD5-3. Do not substitute other diluents. This reconstitution produces a stock solution of 4000 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 200 µL of Calibrator Diluent RD5-3 into each tube. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse Tpo Standard (4000 pg/mL) serves as the high standard. The Calibrator Diluent RD5-3 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

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

1. Prepare all reagents, standards, control and 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 RD1-23 to each well.
4. Add 50 µL of standard, control and prepared sample per well. Mix by gently tapping the plate frame for 1 minute. 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.

   Note: *Samples must be pipetted within 15 minutes.*
5. Aspirate each well and wash, repeating the process four times for a total of five 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 Mouse Tpo 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, control 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 mouse Tpo 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

1. Kaushansky, K. (1996) Leukemia 10 Suppl. 1:s46.
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3. Bartley, T.D. *et al.* (1994) Cell 77:1117.
4. Kaushansky, K. *et al.* (1994) Nature 369:568.
5. Lok, S. *et al.* (1994) Nature 369:565.
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8. Foster, D. and S. Lok (1996) Stem Cells 14 Suppl. 1:102.
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10. Vigon, I. *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:5640.
11. Vigon, I. *et al.* (1993) Oncogene 8:2607.
12. Souyri, M. *et al.* (1990) Cell 63:1137.
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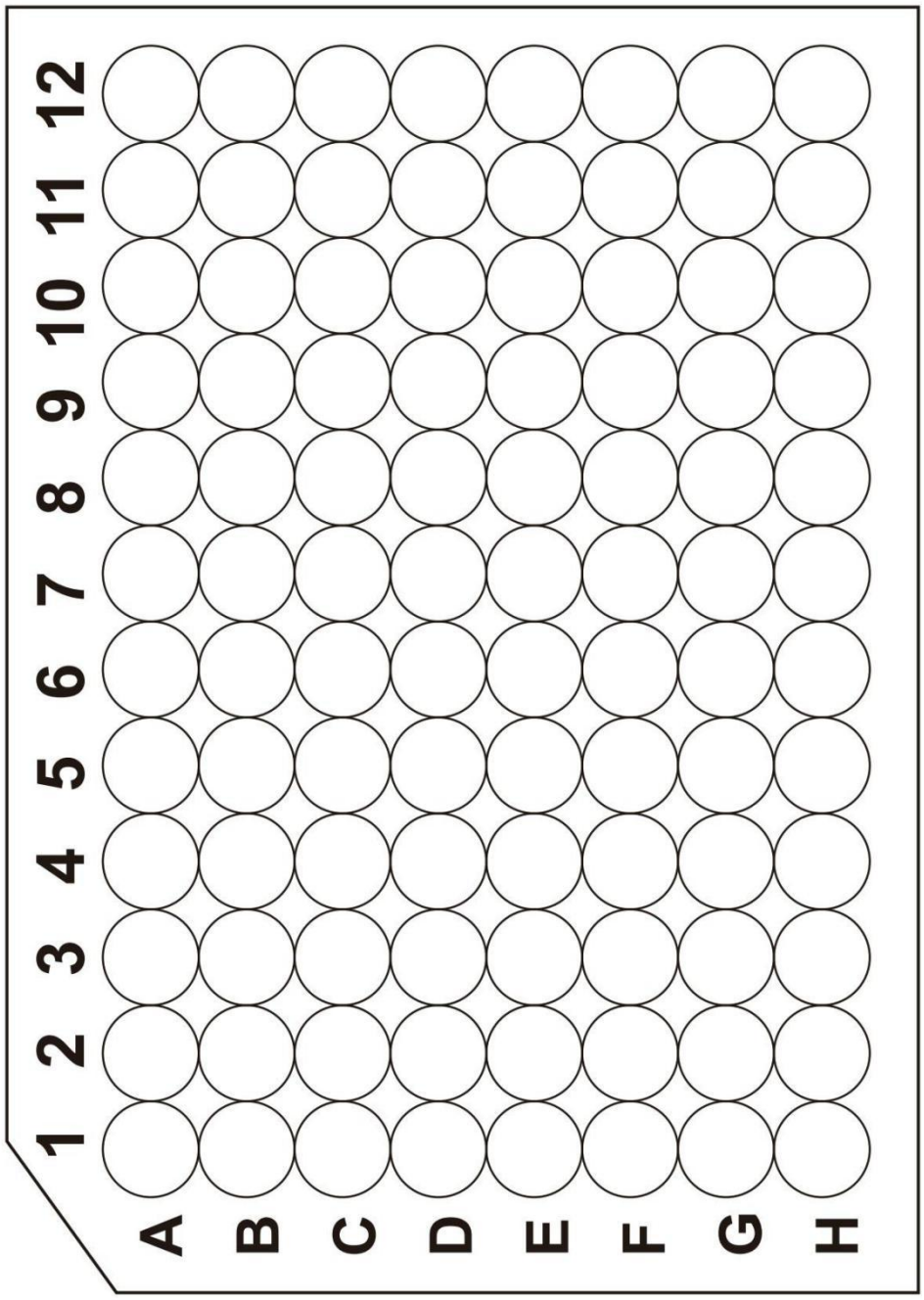
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 Thrombopoietin Valukine™ ELISA 试剂盒

目录号：VAL653

适用于定量检测天然和重组小鼠血小板生成素 (Tpo) 的浓度

科研专用，不可用于临床诊断

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版本号 202506.1

目录

I. 背景

血小板生成素（Tpo），也称为 MGDF，是 *c-Mpl* 原癌基因编码受体的配体，是体外和体内巨核细胞生成和血小板生成的关键调节因子（1）。Tpo 已从包括人、小鼠、大鼠和犬类等多个物种中纯化并克隆出来（2-7）。不同物种的蛋白质在氨基酸（aa）序列水平上具有高度保守性，表现出 69-75%的序列同源性。小鼠 Tpo cDNA 序列编码一个 356 aa 残基的蛋白，其中包含一个 21 aa 残基的信号肽，该信号肽被切割后产生 335 aa 残基的成熟蛋白（3, 5）。成熟的 Tpo 可以分为两个结构域：氨基末端半部分与 Epo 同源，羧基末端部分富含丝氨酸、苏氨酸和脯氨酸残基，并包含七个潜在的 N-糖基化位点。小鼠 Tpo 的 Epo 结构域与小鼠 Epo 的氨基酸序列同源性约为 21%，已被证实能激活 *c-Mpl* 受体。Tpo 的羧基末端结构域已被证明能调节 Tpo 的特异性活性和半衰期。羧基末端还可能在促进 Tpo 的有效生物合成和分泌中发挥作用（8）。已分离出一种 Tpo 的变异形式（Tpo-2）的 cDNA，该变异形式在 Epo 同源结构域内缺失四个氨基酸残基，分泌能力非常差，缺乏 Tpo 活性（9）。Tpo mRNA 主要在成年和胎儿的肝脏、肾脏和骨髓基质细胞中表达（5）。

Tpo 的受体（*c-Mpl*）已从人和小鼠细胞中克隆出来（10, 11）。*c-Mpl* 原癌基因最初被鉴定为一种逆转录病毒癌基因 *v-Mpl* 的细胞同源物（12）。*C-Mpl* 是细胞因子受体超家族的成员。已从小鼠细胞中分离出编码跨膜受体和可溶性受体形式的两个 cDNA 克隆（11）。小鼠 *c-Mpl* 原癌基因已被定位在小鼠第 4 号染色体的 C 带上。MPLV 的 *v-Mpl* 癌基因编码一个融合蛋白，含有与截短的 Tpo 受体融合的逆转录病毒 env 序列（10-12）。Tpo 受体的表达仅限于造血组织。已知表达 Mpl 的细胞包括血小板、巨核细胞和巨核细胞祖细胞（13）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠Tpo抗体包被于微孔板上。样品，质控品和标准品中的小鼠Tpo会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗小鼠Tpo检测抗体进行孵育。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，小鼠血清样本和小鼠血浆样本；
- 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RD5-3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20次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	4	1	2	3
平均值 (pg/mL)	123	455	2364	119	457	2389
标准差	9.8	17.0	58.4	9.6	21.7	115
CV%	8.0	3.7	2.5	8.1	4.7	4.8

B. 回收率

在不同类别样本中掺入检测范围内不同水平的小鼠Tpo，测定其回收率。

样本类型	平均回收率%	范围 (%)
细胞培养基 (n=5)	97	87-115
小鼠血清* (n=5)	100	88-111
小鼠EDTA血浆* (n=5)	104	87-116
小鼠枸橼酸钠血浆* (n=45	104	88-113

*样品在检测前按照样品制备部分的指示进行样本稀释。

C. 灵敏度

小鼠Tpo的最低可检测量（MDD）一般小于 20 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生产的高纯度的NS0表达的重组小鼠Tpo校正。

E. 线性

不同的样本类型（至少含有5个样本）中含有或掺入高浓度的小鼠Tpo，然后用标准品稀释液RD5-3将其稀释并测定其线性。下表所示为样本稀释的结果。

样本种类	稀释倍数	观测值 (pg/mL)	期望值 (pg/mL)	$\frac{\text{观测值}}{\text{期望值}}$ x 100
细胞培养基	Neat	3060	——	——
	1:2	1462	1530	96%
	1:4	759	765	99%
	1:8	358	382	94%
	1:16	169	191	88%
小鼠血清*	Neat	4029	——	——
	1:2	2139	2014	106%
	1:4	1062	1007	105%
	1:8	542	504	108%
	1:16	275	252	109%
小鼠EDTA血浆*	Neat	3805	——	——
	1:2	2012	1902	106%
	1:4	1005	951	106%
	1:8	510	476	107%
	1:16	272	238	114%
小鼠枸橼酸钠血浆*	Neat	3368	——	——
	1:2	1789	1684	106%
	1:4	885	842	105%
	1:8	442	421	105%
	1:16	199	210	95%

*样品在检测前按照样品制备部分的指示进行样本稀释。

F. 样本预值

小鼠血清/血浆样本 - 在此测定中评估样本中小鼠Tpo的存在。

样本类型	平均值 (pg/mL)	范围 (pg/mL)	标准差 (pg/mL)
小鼠血清样本 (n=20)	4627	2972-6645	992
小鼠 EDTA 血浆样本 (n=20)	1771	724-3706	710
小鼠枸橼酸钠血浆样本 (n=20)	1754	841-3544	937

G. 特异性

此 ELISA 法可检测天然及重组小鼠 Tpo。

将以下因子用标准品稀释液 RD5-3 配制成 50 ng/mL 的浓度来检测与小鼠 Tpo 的交叉反应。将 50 ng/mL 的干扰因子掺入中间范围小鼠 Tpo 对照品中，来检测对小鼠 Tpo 的干扰。没有观察到明显的交叉反应或干扰。

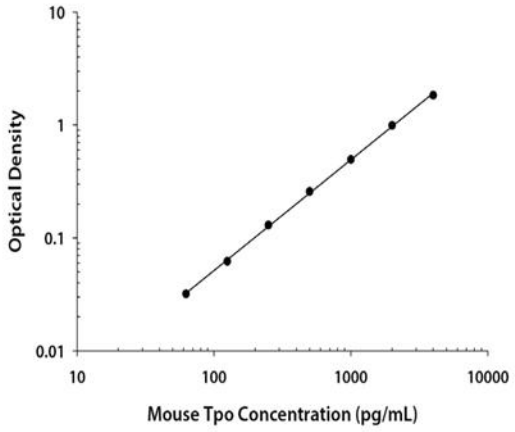
Recombinant mouse:		**Recombinant human:**
C10	IL-10 R	Epo
G-CSF	IL-12	
GM-CSF	IL-13	
IFN-γ	JE/MCP-1	
IL-1α	LIF	
IL-1β	M-CSF	
IL-2	MIP-1α	
IL-3	MIP-1β	
IL-4	MIP-2	
IL-5	SCF	
IL-6	TNF-α	
IL-7	Tpo R	
IL-9	VEGF	
IL-10		

重组人 Tpo（NS0 表达）在本试验中没有干扰，但会有约 0.65%的交叉反应。

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	0.039 0.041	0.040	—
62.5	0.071 0.073	0.072	0.032
125	0.102 0.102	0.102	0.062
250	0.168 0.172	0.170	0.130
500	0.297 0.298	0.298	0.258
1000	0.533 0.535	0.534	0.494
2000	1.024 1.040	1.032	0.992
4000	1.868 1.879	1.874	1.834

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse Tpo Microplate	包被抗小鼠Tpo抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Mouse Tpo Conjugate	酶标检测抗小鼠Tpo抗体	1瓶
Mouse Tpo Standard	小鼠Tpo标准品（冻干），参考瓶身标签进行重溶	1瓶
Mouse Tpo Control	小鼠Tpo 质控品（冻干），质控品的测定值应在标签上规定的范围内	1瓶
Assay Diluent RD1-23	检测液	1瓶
Calibrator Diluent RD5-3	标准品稀释液用于稀释标准品和样本	1瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25×）	1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	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>检测液RD1-23</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品稀释液RD5-3</td></tr>
<tr><td>质控品</td><td rowspan="2">每次使用新鲜配制的质控品和标准品，复溶后 8小时内丢弃。</td></tr>
<tr><td>标准品</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450nm检测波长的吸收值及540nm或570nm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
- 500mL量筒
- 用于稀释标准品和样品的管子

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. 样本收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样本稳定性尚未评估。

细胞培养上清：颗粒物应通过离心去除；立即检测样本或分装，≤ -20 °C储存备用，避免反复冻融。样本可能需要用标准品稀释液RD5-3稀释。

血清样本：血液样本在室温下凝集2小时，然后在2000 × g下离心20分钟。吸取血清样本之后即刻用于检测，或者分装，≤ -20°C储存备用。避免反复冻融。样本可能需要用标准品稀释液RD5-3稀释。

血浆样本：使用 EDTA 或枸橼酸钠作为抗凝剂收集血浆。然后2000 × g离心20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储存备用。避免反复冻融。样本可能需要用稀释液RD5-3稀释。

注意：*本试剂盒不推荐用于肝素血浆。*

严重溶血或血脂样本不适合用于本试剂盒。

B. 样本准备工作

小鼠血清及血浆样本建议用标准品稀释液RD5-3 5倍稀释后进行检测，即50µL血清+200 µL标准品稀释液RD5-3。混合均匀并使样本在检测前平衡20分钟。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。

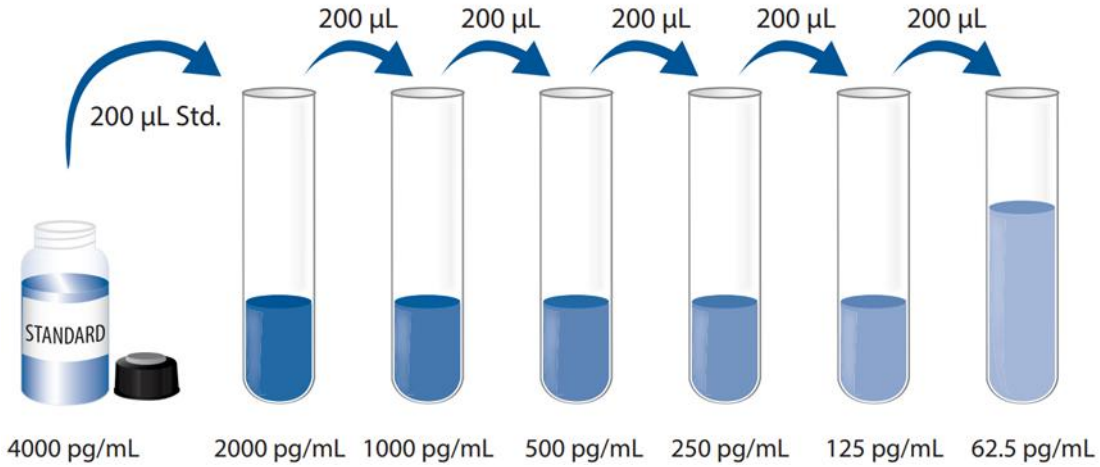
小鼠Tpo质控品：使用1.0 mL去离子水或蒸馏水重溶质控品。混合均匀，测定时不稀释质控品。

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×）。

小鼠Tpo标准品：重溶体积请参考瓶身标签*，用标准品稀释液RD5-3重溶小鼠Tpo标准品，不使用其他稀释液。得到浓度为4000 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。

向每个稀释管中加入200 µL标准品稀释液RD5-3。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。未稀释的小鼠Tpo标准品可用作标准曲线最高点（4000 pg/mL），标准品稀释液RD5-3可用作标准曲线零点（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，质控品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，质控品和样本；
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. 每孔加入50μL检测液RD1-23。
4. 分别将不同浓度标准品，质控品和实验样本加入相应孔中，每孔50 μL。轻轻拍打微孔板1分钟，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；

   注意：*样本须在15分钟内完成上样。*
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 μL，然后将板内洗涤液吸去。重复操作4次，共洗5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小鼠Tpo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，质控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。数据可以通过绘制小鼠Tpo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