# PRODUCT INFORMATION & MANUAL

## Mouse Erythropoietin (Epo) Valukine™ ELISA

## Catalog Number: VAL643

For the quantitative determination of natural and recombinant mouse Erythropoietin (Epo) 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

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# I. BACKGROUND

Erythropoietin (Epo) is a 34-39 kDa secreted glycoprotein that is a member of the type I cytokine superfamily. The mouse Epo gene encodes a 192 amino acid (aa) residue precursor that contains a 26 aa signal peptide and a 166 aa mature protein containing three potential N-linked glycosylation sites (1-4). Mouse Epo lacks the O-linked glycosylation site found in human Epo. Although carbohydrate chains are not required for *in vitro* receptor binding, they are required for *in vivo* Epo bioactivity. Depending on the cell source, different Epo isoforms are produced that differ in their glycan compositions and sialic acid contents (5-8). Mature mouse and rat Epo share 94% aa sequence identity. They also share from 80%-82% aa identity with mature human, porcine, rhesus monkey and feline Epo (2, 3). Epo is primarily produced by cells in the kidney (interstitial peritubular renal fibroblasts) and liver (hepatocytes and Ito cells), where its production is up-regulated by hypoxia. Other tissues and cells, including neural tissues (astrocytes and neurons), testis (Sertoli cells), uterus, placenta, and erythroid progenitors, have also been shown to produce Epo (9-14).

Epo is best known for its role in red blood cell formation. While Epo is not a lineage commitment factor, it inhibits apoptosis and induces burst forming unit-erythroid (BFU-E) differentiation into colony forming unit-erythroid (CFU-E), and the subsequent proliferation and maturation of CFU-E into early normoblasts (10, 15, 16). Apart from its role in erythropoiesis, Epo also acts on various non-hematopoietic cells to function as a viability and proliferation factor. Epo can stimulate myoblast proliferation while suppressing its differentiation, resulting in the expansion of the progenitor cell population (17). Epo is a tissue- protective factor that protects against ischemic and toxic injuries to neuronal, cardiovascular and renal tissues (18, 19). Epo has also been shown to promote angiogenesis in various physiologic and pathologic conditions (20, 21).

Epo binds and signals via the high-affinity preformed homodimeric Epo receptor (Epo R) that is composed of two Epo R subunits. Each Epo R subunit is a type I transmembrane glycoprotein that belongs to the type I cytokine receptor superfamily (18, 22-24). Its extracellular domain contains the characteristic two fibronectin type III domains and a WSxWS motif near the plasma membrane (24, 25). Binding of Epo to the Epo R homodimer results in conformational change and phosphorylation and activation of the non-receptor protein kinase JAK2, which activates the downstream signaling cascade (26). An alternative Epo heteromeric receptor complex that transduces cell-protective signals and containing the β common receptor (βCR) subunit in addition to the Epo R subunit has been described. βCR also belongs to the type I cytokine receptor superfamily and is a subunit that is shared by the heteromeric IL-3, IL-5 and GM-CSF receptor complexes. Epo binds with lower affinity to the heteromeric receptor consisting of a Epo R subunit and a βCR homodimer (18).

# II. OVERVIEW

## A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse Epo has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse Epo present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse Epo 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 Epo 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, tissue homogenates, mouse serum and mouse 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 RD6Z 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) | 196 | 258 | 704 | 180 | 239 | 700 |
| Standard Deviation | 7.7 | 7.8 | 31.2 | 17.5 | 15.3 | 17.5 |
| CV% | 3.9 | 3.0 | 4.4 | 9.7 | 6.4 | 2.5 |

## B. RECOVERY

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

| Sample Type | Average % Recovery | Range (%) |
|---|---|---|
| Cell culture media (n=7) | 110 | 102-117 |
| Tissue homogenates (n=3) | 94 | 80-111 |
| Mouse serum* (n=9) | 93 | 85-114 |
| Mouse heparin plasma* (n=8) | 103 | 90-120 |

* Mouse serum and plasma samples were spiked and then diluted as described in the Sample Preparation section.

## C. SENSITIVITY

Nineteen assays were evaluated and the minimum detectable dose (MDD) of mouse Epo ranged from 6.5-46.9 pg/mL. The mean MDD was 18.0 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 *Sf* 21-expressed recombinant mouse Epo 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 Epo and diluted with Calibrator Diluent RD6Z to produce samples with values within the dynamic range of the assay.

| | | **Cell culture supernates (n=9)** | **Tissue homogenates (n=3)** | **Mouse serum* (n=6)** | **Mouse heparin plasma* (n=9)** |
|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 92 | 106 | 95 | 94 |
| | Range (%) | 90-96 | 94-120 | 91-98 | 89-97 |
| 1:4 | Average % of Expected | 93 | 105 | 96 | 96 |
| | Range (%) | 90-102 | 92-119 | 90-100 | 88-102 |
| 1:8 | Average % of Expected | 94 | 107 | 98 | 97 |
| | Range (%) | 90-100 | 94-120 | 90-105 | 87-106 |
| 1:16 | Average % of Expected | 95 | 106 | 106 | 97 |
| | Range (%) | 85-103 | 95-113 | 95-117 | 83-108 |

* Mouse serum and plasma samples were spiked and then diluted as described in the Sample Preparation section.

## F. SAMPLE VALUES

**Mouse serum/plasma -** Samples were evaluated for detectable levels of mouse Epo in this assay.

| Sample Type | Mean of Detectable (pg/mL) | % Detectable | Range (pg/mL) |
|---|---|---|---|
| Mouse serum (n=20) | 237 | 65 | ND-683 |
| Mouse heparin plasma (n=20) | 151 | 30 | ND-198 |

ND=Non-detectable

**Tissue Homogenates -** Lung, heart, and spleen tissues from three adult female mice were prepared as described in the Sample Collection and Storage section. Supernates were removed, tested for mouse Epo, and measured 61 pg/mL, 111 pg/mL, and 75 pg/mL, respectively.

## G. SPECIFICITY

This assay recognizes natural and recombinant mouse Epo.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD6Z and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse Epo control were assayed for interference. No significant cross-reactivity or interference was observed.

| **Recombinant mouse:** | | |
|---|---|---|
| ICAM-1 | IL-6 | Leptin |
| IFN-γ | IL-7 | LIF |
| IGF-I | IL-9 | M-CSF |
| IGF-II | IL-10 | MIP-1α |
| IL-1α | IL-11 | MIP-1β |
| IL-1β | IL-12 | OSM |
| IL-1ra | IL-12 p40 | RANTES |
| IL-2 | IL-13 | TNF RII |
| IL-3 | IL-17 | TNF-α |
| IL-4 | IL-18 | Tpo |
| IL-5 | JE/MCP-1 | VEGF |

Recombinant mouse Epo R/Fc Chimera interferes at concentrations > 2.0 ng/mL in this assay.

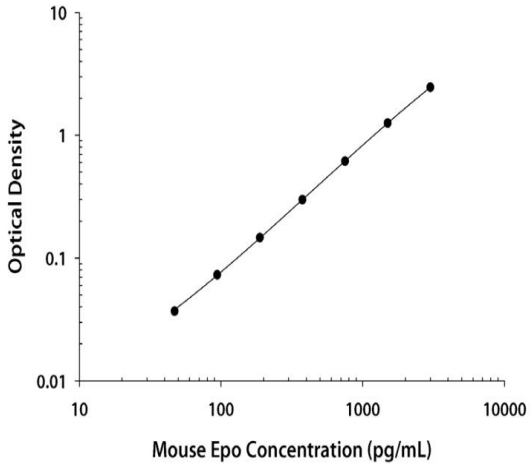
Recombinant human Epo has approximately 9% cross-reactivity in this assay at concentrations ≥ 2.0 ng/mL.

Recombinant rat Epo has 100% cross-reactivity in this assay. This assay is not validated for use with rat samples because rat sample dilution is not linear.

# 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 | 0.041<br>0.045 | 0.043 | — |
| 47 | 0.078<br>0.082 | 0.080 | 0.037 |
| 94 | 0.113<br>0.118 | 0.116 | 0.073 |
| 188 | 0.187<br>0.192 | 0.190 | 0.147 |
| 375 | 0.339<br>0.345 | 0.342 | 0.299 |
| 750 | 0.658<br>0.660 | 0.659 | 0.616 |
| 1500 | 1.284<br>1.314 | 1.299 | 1.256 |
| 3000 | 2.473<br>2.521 | 2.497 | 2.454 |

# V. KIT COMPONENTS AND STORAGE

## A. MATERIALS PROVIDED

| Parts | Description | Size |
|---|---|---|
| Mouse Epo Microplate | 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse Epo. | 1 plate |
| Mouse Epo Conjugate | An antibody specific for mouse Epo conjugated to horseradish peroxidase. | 1 vial |
| Mouse Epo Standard | Recombinant mouse Epo in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume. | 1 vial |
| Mouse Epo Control | Recombinant mouse Epo 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 RD1W | A buffered protein solution. | 1 vial |
| Calibrator Diluent RD6Z | A buffered protein solution 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 vial |
| 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="6">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Assay Diluent RD1W</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Calibrator Diluent RD6Z</td></tr>
<tr><td>Control</td><td rowspan="2">Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze- thaw cycles.</td></tr>
<tr><td>Standard</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 (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- 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 RD6Z.

**Tissue Homogenates** - The preparation of tissue homogenates will vary depending upon the tissue type. For this assay, heart, lung, and spleen tissue from three mice was rinsed with 1 x PBS to remove excess blood, homogenized in 5-10 mL of 1 x PBS, and stored overnight at ≤ -20 °C. After two freeze-thaw cycles to break up the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. Homogenates should be assayed immediately or aliquotted and stored at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD6Z.

**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 RD6Z.

**Plasma -** Collect plasma using heparin 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 RD6Z.

***Note:*** *EDTA and citrate plasma has not been validated 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 2-fold dilution into Calibrator Diluent RD6Z prior to assay. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD6Z. Optimal dilutions should be determined by the end user.

## C. REAGENT PREPARATION

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

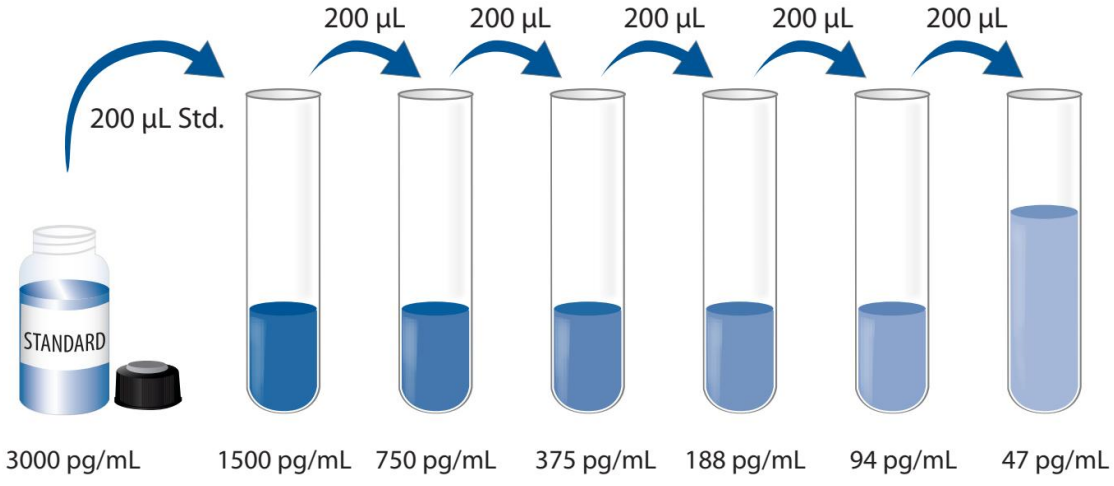
**Mouse Epo Control** - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

**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 Epo Standard- Refer to the vial label for the reconstitution volume*** Reconstitute the Mouse Epo Standard with Calibrator Diluent RD6Z. Do not substitute other diluents. This reconstitution produces a stock solution of 3000 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 RD6Z into each tube.** Use the standard stock solution to produce a dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse Epo Standard (3000 pg/mL) serves as the high standard. The Calibrator Diluent RD6Z 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, standard dilutions, 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 RD1W to each well.
4. Add 50 µL of standard, control and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.** A plate layout is provided for a record of standards and samples assayed.
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 Epo Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.**
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 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 Epo 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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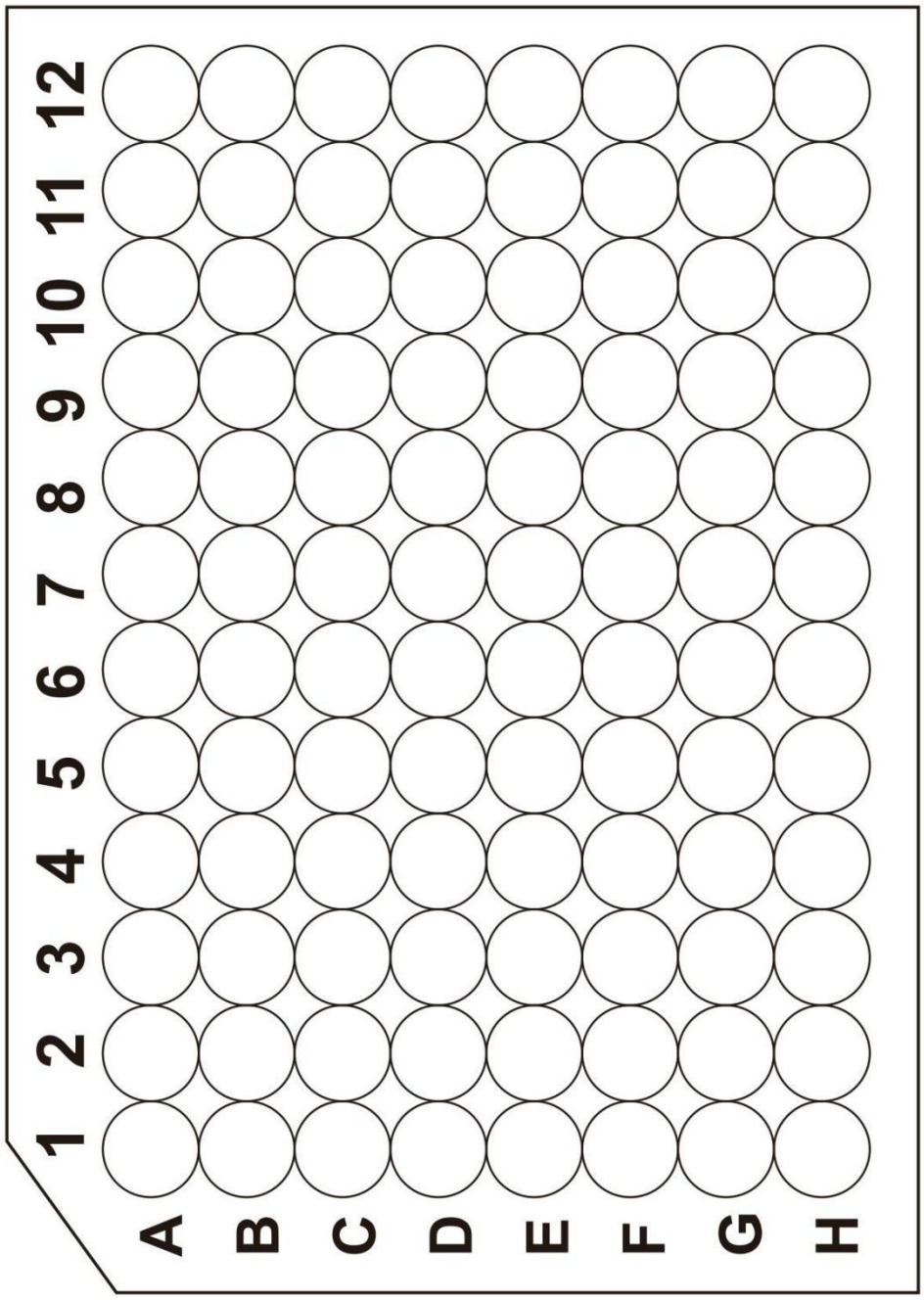
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



# 产品信息及操作手册

## 小鼠红细胞生成素（Epo）Valukine™ ELISA 试剂盒

**目录号：VAL643**

适用于定量检测天然和重组小鼠红细胞生成素（Epo）的浓度

科研专用，不可用于临床诊断

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# 目录

# I. 背景

红细胞生成素（Erythropoietin，Epo）是一种 34-39 kDa 的分泌型糖蛋白，属于 I 型细胞因子超家族。小鼠 Epo 基因编码 192 个氨基酸（amino acid，aa）残基的前体，其中含有 26 aa 的信号肽和 166 aa 的成熟蛋白，成熟蛋白含有三个潜在的 N 连接糖基化位点（1-4）。小鼠 Epo 缺乏人类 Epo 中的 O 型糖基化位点。虽然体外受体结合不需要碳水化合物链，但它们是体内 Epo 生物活性所必需的。根据细胞来源的不同，会产生不同的 Epo 异构体，它们的聚糖成分和唾液酸含量也不同（5-8）。成熟的小鼠和大鼠 Epo 有 94%的 aa 序列一致性。它们还与成熟的人类、猪、恒河猴和猫 Epo 也有 80%-82%的 aa 相同性（2, 3）。Epo 主要由肾脏（间质周围肾成纤维细胞）和肝脏（肝细胞和 Ito 细胞）中的细胞产生，缺氧会上调这些细胞的生成。其他组织和细胞，包括神经组织（星形胶质细胞和神经元）、睾丸（Sertoli 细胞）、子宫、胎盘和红细胞祖细胞，也能产生 Epo（9-14）。

Epo 因其在红细胞形成过程中的作用而为人熟知。虽然 Epo 并非谱系承诺因子，但它能抑制细胞凋亡，诱导红细胞爆发形成单位（burst forming unit-erythroid，BFU-E）分化为红细胞集落形成单位（colony forming unit-erythroid，CFU-E），并随后使 CFU-E 增殖和成熟为早期正常红细胞（10,15,16）。除了在红细胞生成过程中发挥作用外，Epo 还可作为活力和增殖因子作用于各种非造血细胞。Epo 可刺激肌母细胞增殖，同时抑制其分化，从而导致祖细胞群的扩张（17）。Epo 是一种组织保护因子，可保护神经元、心血管和肾组织免受缺血和毒性损伤（18,19）。研究还表明，Epo 在各种生理和病理条件下都能促进血管生成（20,21）。

Epo 通过由两个 Epo R 亚基组成的高亲和力预成同源二聚体 Epo 受体（Epo R）结合并发出信号。每个 Epo R 亚基都是 I 型跨膜糖蛋白，属于 I 型细胞因子受体超家族（18,22-24）。它的胞外结构域包含两个特征性的纤连蛋白 III 型结构域和一个靠近质膜的 WSxWS 基序（24,25）。Epo 与 Epo R 同源二聚体结合会导致构象变化、磷酸化以及非受体蛋白激酶 JAK2 的激活，从而激活下游信号级联反应（26）。一种可传递细胞保护信号的替代性 Epo 异构受体复合物已被描述，该复合物除 Epo R 亚基外，还含有β共受体（βCR）亚基。βCR 也属于 I 型细胞因子受体超家族，是 IL-3、IL-5 和 GM-CSF 受体异构体复合物共有的亚基。Epo 与由 Epo R 亚基和βCR 同源二聚体组成的异源受体结合的亲和力较低（18）。

# II. 概述

## A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠Epo抗体包被于微孔板上，样品，质控品和标准品中的小鼠Epo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Epo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，组织匀浆样本，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RD6Z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 | 3 | 1 | 2 | 3 |
| 平均值 (pg/mL) | 196 | 258 | 704 | 180 | 239 | 700 |
| 标准差 | 7.7 | 7.8 | 31.2 | 17.5 | 15.3 | 17.5 |
| CV% | 3.9 | 3.0 | 4.4 | 9.7 | 6.4 | 2.5 |

### B. 回收率

在不同类型样本中掺入检测范围内不同水平的小鼠Epo，测定其回收率。

| 样本类型 | 平均回收率% | 范围 (%) |
|---|---|---|
| 细胞培养基 (n=7) | 110 | 102-117 |
| 组织匀浆（n=3） | 94 | 80-111 |
| 小鼠血清* (n=9) | 93 | 85-114 |
| 小鼠肝素血浆* (n=8) | 103 | 90-120 |

*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

19次检测结果表明，小鼠Epo的最低可测剂量（MDD）范围为6.5- 46.9 pg/mL。平均MDD为18.0 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*Sf* 21表达的重组小鼠Epo校正。

## E. 线性

不同的样本中含有或掺入高浓度的小鼠Epo，然后用标准品稀释液RD6Z将样本稀释到检测范围内，测定其线性。

| 稀释倍数 | | 细胞培养上清 (n=9) | 组织匀浆* (n=3) | 小鼠血清* (n=6) | 小鼠肝素血浆* (n=9) |
|---|---|---|---|---|---|
| 1:2 | 平均值/期待值（%） | 92 | 106 | 95 | 94 |
| | 范围（%） | 90-96 | 94-120 | 91-98 | 89-97 |
| 1:4 | 平均值/期待值（%） | 93 | 105 | 96 | 96 |
| | 范围（%） | 90-102 | 92-119 | 90-100 | 88-102 |
| 1:8 | 平均值/期待值（%） | 94 | 107 | 98 | 97 |
| | 范围（%） | 90-100 | 94-120 | 90-105 | 87-106 |
| 1:16 | 平均值/期待值（%） | 95 | 106 | 106 | 97 |
| | 范围（%） | 85-103 | 95-113 | 95-117 | 83-108 |

*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-** 在此测定中对样品进行小鼠Epo的可检测水平的评估。

| 样本类型 | 可检测平均值 (pg/mL) | 可检测率 % | 范围(pg/mL) |
|---|---|---|---|
| 小鼠血清样本 (n=20) | 237 | 65 | ND-683 |
| 小鼠肝素血浆样本 (n=20) | 151 | 30 | ND-198 |

ND=未检测到

**组织匀浆 -** 三只成年雌性小鼠的肺、心脏和脾脏组织按照样本收集和储存部分中的描述制备。取出上清液检测小鼠 Epo，结果分别为 61 pg/mL、111 pg/mL 和 75 pg/mL。

## G. 特异性

此ELISA法可检测天然和重组小鼠Epo。

将以下因子用标准品稀释液RD6Z配制成 50 ng/mL的浓度来检测与小鼠Epo的交叉反应。将 50 ng/mL的干扰因子掺入中间范围的重组小鼠Epo对照品中，来检测对小鼠Epo的干扰。没有观察到明显的交叉反应或干扰。

| **Recombinant mouse:** | | |
|---|---|---|
| ICAM-1 | IL-6 | Leptin |
| IFN-γ | IL-7 | LIF |
| IGF-I | IL-9 | M-CSF |
| IGF-II | IL-10 | MIP-1α |
| IL-1α | IL-11 | MIP-1β |
| IL-1β | IL-12 | OSM |
| IL-1ra | IL-12 p40 | RANTES |
| IL-2 | IL-13 | TNF RII |
| IL-3 | IL-17 | TNF-α |
| IL-4 | IL-18 | Tpo |
| IL-5 | JE/MCP-1 | VEGF |

重组小鼠Epo R/Fc嵌合体在浓度> 2.0 ng/mL时存在干扰。

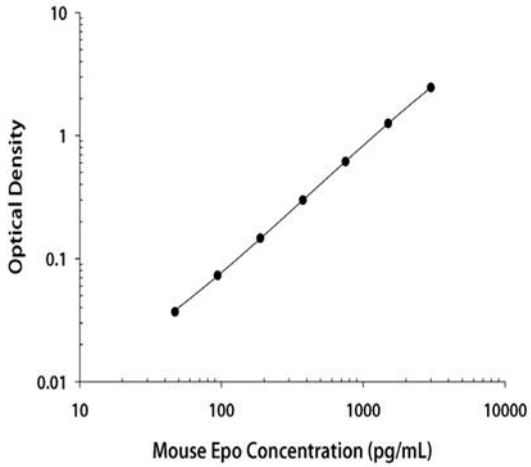
重组人Epo在浓度≥2.0 ng/mL时具有约9%的交叉反应性。。

重组大鼠 Epo 在本检测试剂盒中具有 100% 的交叉反应性。由于大鼠样本稀释不呈线性，因此该试剂盒未对大鼠样本进行验证。

# 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 | 0.041<br>0.045 | 0.043 | — |
| 47 | 0.078<br>0.082 | 0.080 | 0.037 |
| 94 | 0.113<br>0.118 | 0.116 | 0.073 |
| 188 | 0.187<br>0.192 | 0.190 | 0.147 |
| 375 | 0.339<br>0.345 | 0.342 | 0.299 |
| 750 | 0.658<br>0.660 | 0.659 | 0.616 |
| 1500 | 1.284<br>1.314 | 1.299 | 1.256 |
| 3000 | 2.473<br>2.521 | 2.497 | 2.454 |

# V. 试剂盒组成及储存

## A. 试剂盒组成

| 组成 | 描述 | 规格 |
|---|---|---|
| Mouse Epo Microplate | 包被抗小鼠Epo抗体的96孔聚苯乙烯板，8孔× 12条 | 1块板 |
| Mouse Epo Conjugate | 酶标检测抗小鼠Epo抗体 | 1瓶 |
| Mouse Epo Standard | 小鼠Epo标准品（冻干），参考瓶身标签进行重溶 | 1瓶 |
| Mouse Epo Control | 小鼠Epo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 RD1W | 检测液 | 1瓶 |
| Calibrator Diluent RD6Z | 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RD1W</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RD6Z</td></tr>
<tr><td>质控品</td><td rowspan="2">等分并在 ≤ -20 °C的冰箱中最多储存30天*。避免反复冻融</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
- 水平振荡器（0.12” 轨道），转速：500±50 rpm
- 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：**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RD6Z稀释。

**组织匀浆样本：**组织匀浆的制备因组织类型而异。在本试验中，用1 × PBS冲洗3只小鼠的心脏、肺和脾脏组织以去除多余的血液，在5-10 mL 的1 × PBS中匀浆，并在≤ -20 °C 下保存过夜。经过2次冻融循环使细胞膜破碎后，匀浆液在5000 × g下离心5分钟。匀浆应立即检测或者分装，并在 ≤ -20 °C储存备用。避免反复冻融。样品可能需要用标准品稀释液 RD6Z 稀释。

**血清样本：**血液样品在室温下凝集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（1×） 稀释。

**血浆样本：**使用肝素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。避免反复冻融循环。样品可能需要用标准品稀释液 RD6Z 稀释。

***注意：*** *本试剂盒对枸橼酸钠和EDTA血浆尚未被验证。*

*本试剂盒对严重溶血或血脂样品可能不适合使用。*

## B. 样本准备工作

小鼠血清样本和血浆样本建议用标准品稀释液RD6Z 2倍稀释后进行检测，即70 µL血清+70 µL标准品稀释液RD6Z。即是2倍稀释。 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。**

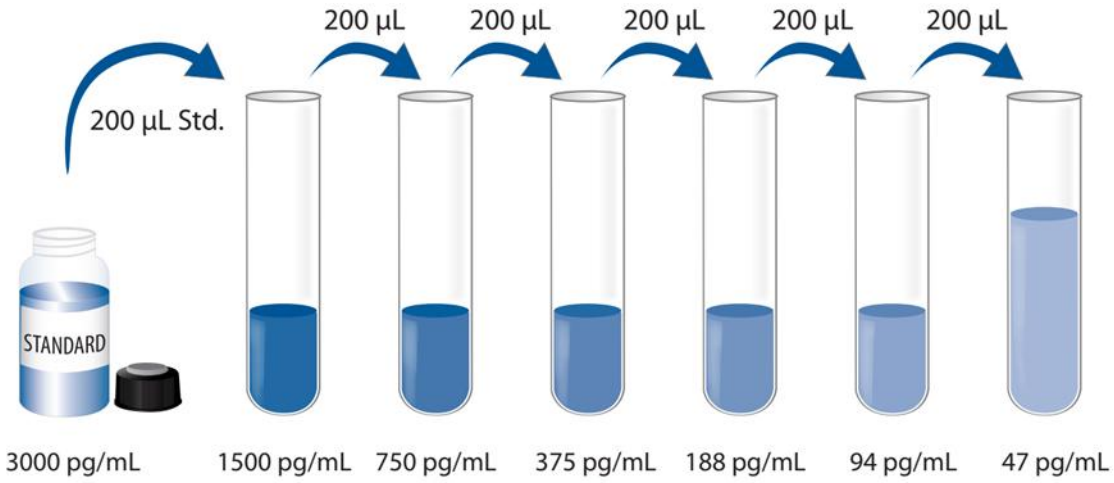
**小鼠Epo质控品：**使用1.0 mL去离子水或蒸馏水重溶质控品。混合均匀，测定时不稀释质控品。

**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Epo标准品：**重溶体积请参考瓶身标签*，用标准品稀释液RD6Z重溶小鼠Epo标准品。请勿用其他稀释液替代。得到浓度为3000 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。

**每个稀释管中加入200 µL标准品稀释液RD6Z。**将标准品母液参照下图做系列稀释，每管须充分且缓慢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（3000 pg/mL），标准品稀释液RD6Z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RD1W。
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。用封板膜封住反应孔，**在水平振荡器（0.12”轨道）上，转速：500±50 rpm，室温孵育2小时.**说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 μL，然后将板内洗涤液吸去。重复操作4次，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Epo酶标检测抗体。用封板膜封住反应孔，**在水平振荡器（0.12”轨道）上，转速：500±50 rpm，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Epo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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## 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