PRODUCT INFORMATION & MANUAL

Mouse alpha-Fetoprotein/AFP Valukine™ ELISA

VAL622

For the quantitative determination of natural and recombinant mouse alpha-Fetoprotein/AFP 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

Version202209.1

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I. BACKGROUND

α-Fetoprotein (AFP), a member of the albuminoid superfamily (Albumin, Vitamin D-binding protein, and α-Albumin), is a fetal- and tumor-associated protein well known as a marker for certain cancers and congenital defects (1-4). In humans and rodents, it is a single chain glycoprotein of 66-72 kDa, with 3-5% of the molecular weight resulting from glycosylation (3). Mouse AFP shares 81% and 65% amino acid sequence identity with rat and human AFP, respectively. AFP is synthesized in the fetus primarily by the liver, yolk sac, and tissues of gastrointestinal origin (5-8). During mouse development, AFP is expressed at low but detectable levels in the foregut endoderm, becomes highly activated in hepatoblasts that form the liver bud, and is one of the earliest markers of the hepatocyte lineage (9). Fetal serum levels peak at weeks 10 through 13 and then decline throughout gestation (10). In contrast, maternal serum AFP, derived primarily from fetal circulation, continues to increase into the third trimester before declining (10).

AFP can act as a carrier protein, binding several ligands including steroids, bilirubin, fatty acids, retinoids, and flavonoids (4). In rodents, AFP sequesters endogenous estrogen and blocks the induction of estrogen receptor (ER)-mediated responses (2). Ligand binding may affect the rigidity and conformation of the AFP tertiary structure (2, 4, 11, 12). In addition, AFP has been proposed to have immunosuppressive activity, regulate cell proliferation and apoptosis, initiate intracellular signaling, and contribute to cell invasion (4, 13). Putative cell surface receptors for AFP have been described, and AFP is internalized by many cell types (14). AFP knockout mice are viable, but females are infertile due to an inadequate hormonal environment that blocks normal ovulation (15, 16).

Altered levels of both fetal and maternal AFP have been associated with several congenital abnormalities including hypothyroidism, autoimmune disorders, and heart defects (2, 17-19). In addition, AFP is widely used as a prenatal screening marker for neural tube defects and chromosomal abnormality (2, 20). Low maternal serum AFP levels have been associated with a higher incidence of Down's syndrome, whereas higher levels are associated with spina bifida and anencephaly (21-24). Postnatal production of AFP is associated with several pathologies. AFP is a commonly used marker for hepatocellular cancer, and elevated AFP levels are coincident with several other cancers including hepatoblastoma, germ cell tumors, and certain gastric cancers (25-31).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse AFP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse AFP present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for mouse AFP 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 is added to the wells and color develops in proportion to the amount of AFP bound in the initial step. The color development is stopped, and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernate and 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 Reagent 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)	1947.6	504.8	124.5	2086.3	601.6	133.3
Standard Deviation	81.9	20.9	5.6	186.7	38.2	12.8
CV%	4.2	4.1	4.5	8.9	7.2	9.6

B. RECOVERY

The recovery of mouse AFP spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 106.9-129.3% with an average of 118.3%.

The recovery of mouse AFP spiked to different levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 87.0-113.7% with an average of 101.3%.

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse AFP is typically less than 1.23 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 AFP 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 AFP and diluted with Reagent Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	93.6	89.2-95.9
1:4	92.9	85.1-103.7
1:8	91.9	83.0-105.5
1:16	93.7	85.7-110.2

F. SAMPLE VALUES

Serum - Five mouse serum samples were evaluated for the presence of mouse AFP in this assay. All samples measured ranged from 84.4 to 290.7 ng/mL with an average of 197.9 ng/mL.

G. SPECIFICITY

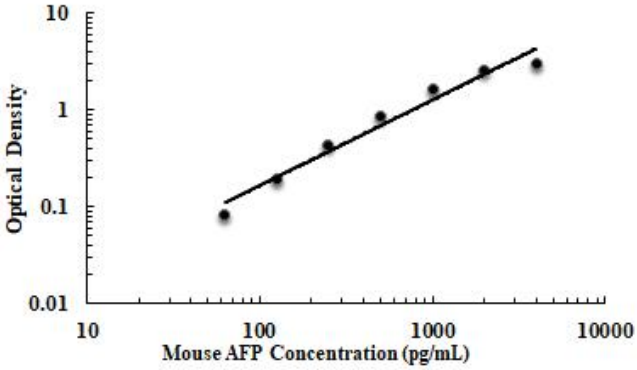
The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant mouse	**Recombinant human**
Leptin	α-Fetoprotein
Prolactin	**Natural proteins**
Resistin	human α-Fetoprotein
Vitamin D BP	mouse serum albumin

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	0.080 0.082	0.081	-
62.5	0.162 0.168	0.165	0.084
125	0.264 0.286	0.275	0.194
250	0.493 0.532	0.513	0.432
500	0.900 0.989	0.945	0.864
1000	1.642 1.757	1.700	1.619
2000	2.517 2.653	2.585	2.504
4000	3.003 3.055	3.029	2.948

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse AFP Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a rat antibody against mouse AFP.	1 plate
Mouse AFP Standard	Recombinant mouse AFP in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Mouse AFP Detection Antibody	Biotinylated AFP antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Reagent Diluent Concentrate (10×)	Concentrated buffered diluent used to dilute standard, samples, Detection Antibody and 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="7">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.</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>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.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500±50 rpm.
- 500 mL graduated cylinder.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear 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 may require dilution with Reagent 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 may require dilution with Reagent Diluent (1×).

B. SAMPLE PREPARATION

Serum samples require a 100-fold dilution. For example, add 10 µL of sample into a tube with 90 µL Reagent Diluent (1×) to prepare a 10-fold diluted sample. Mix through and then pipette 10 µL of prepared 10-fold diluted sample into a tube with 90 µL Reagent Diluent (1×) to prepare a final 100-fold diluted sample.

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×).

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

Detection Antibody- Centrifuge briefly before opening. Reconstitution volume refer to vial label with Reagent Diluent (1×). Aliquot and store if needed. Dilute stock solution in Reagent Diluent (1×) to the working concentration of 200 ng/mL. Prepare at least 15 minutes prior to use.

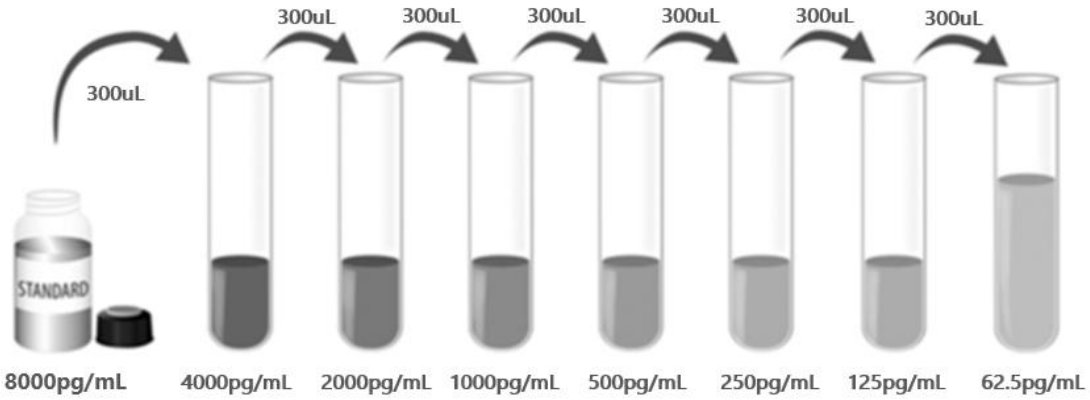
Streptavidin-HRP A (1×) - Centrifuge briefly before opening. Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Mouse AFP Standard - Centrifuge briefly before opening. Refer to the vial label

for the reconstitution volume*. This reconstitution produces a stock solution of 8000 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 Reagent Diluent (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. The Reagent 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, or prepared sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature **on a horizontal orbital microplate shaker set at 500±50rpm.** 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 diluted in Reagent Diluent (1×), 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±50rpm.**
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 20 minutes at room temperature **on a horizontal orbital microplate shaker set at 500±50rpm. Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate to each well. Incubate for 20 minutes at room temperature **on a horizontal orbital microplate shaker set at 500±50rpm. 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, 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 AFP 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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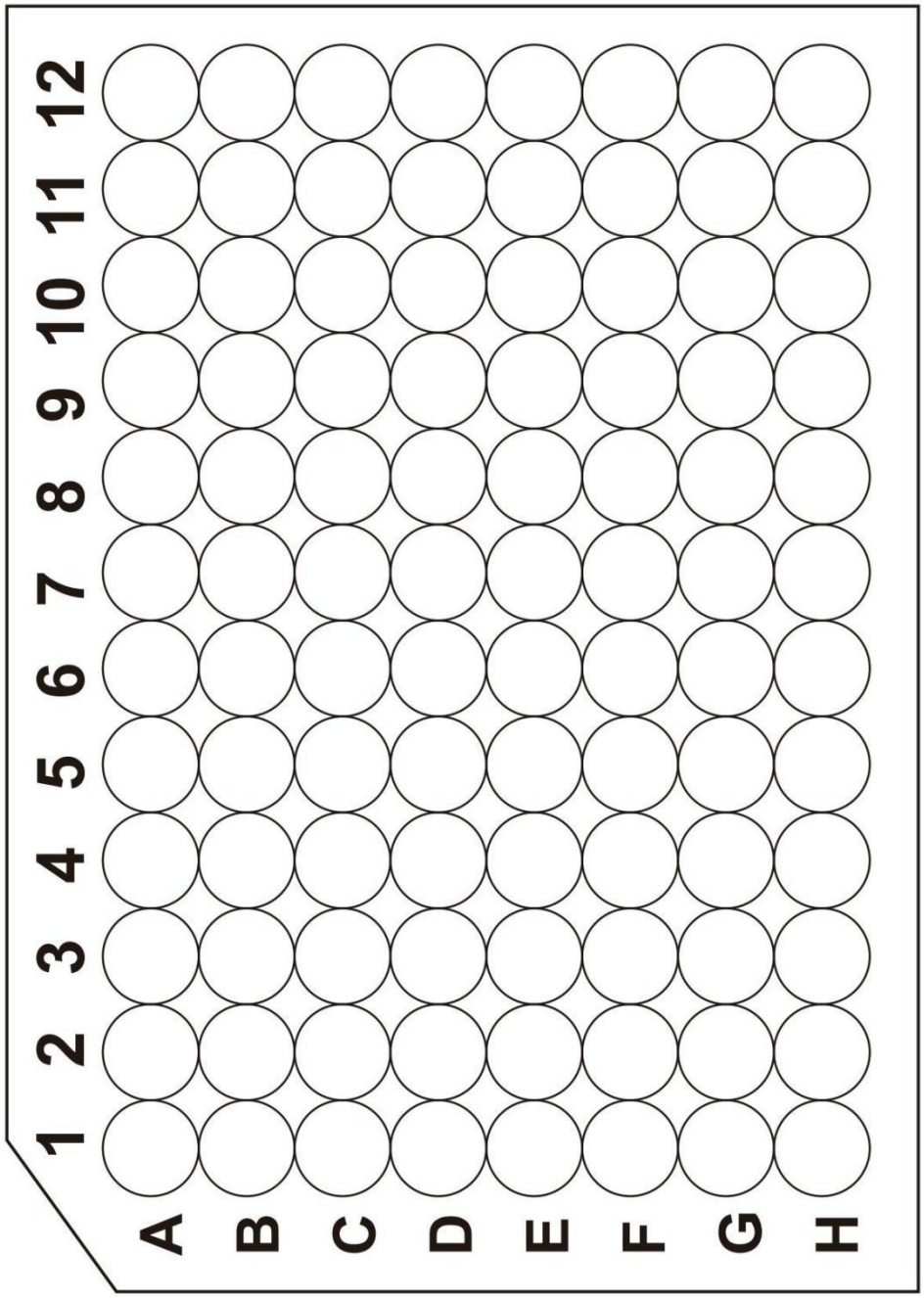
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 alpha-Fetoprotein/AFP Valukine™ ELISA 试剂盒

目录号：VAL622

适用于定量检测天然和重组小鼠 alpha-Fetoprotein/AFP 的浓度

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目录

I. 背景

α-甲胎蛋白（AFP）是类白蛋白超家族（白蛋白、维生素 D 结合蛋白和 α-白蛋白）的一员，是一种胎儿和肿瘤相关蛋白，被认为是某些癌症和先天性缺陷的标志物（1-4）。在人类和啮齿类动物中，它是一种 66-72 kDa 的单链糖蛋白，3-5%的分子量由糖基化产生（3）。小鼠 AFP 与大鼠、人 AFP 的氨基酸序列同源性分别为 81%和 65%。AFP 在胎儿中主要由肝脏、卵黄囊和胃肠道来源的组织合成（5-8）。在小鼠发育过程中，AFP 在前肠内胚层中的表达水平较低但可检测到，在形成肝芽的肝母细胞中高度激活，是肝细胞谱系的最早标记之一（9）。胎儿血清水平在第 10 至 13 周达到峰值，然后在整个妊娠期下降（10）。相比之下，主要来源于胎儿循环的母体血清 AFP 在妊娠晚期继续增加，然后下降（10）。

AFP 可以作为载体蛋白，结合多种配体，包括类固醇、胆红素、脂肪酸、维甲酸和类黄酮（4）。在啮齿类动物中，甲胎蛋白可隔离内源性雌激素，并阻断雌激素受体（ER）介导的反应的诱导（2）。配体结合可能影响 AFP 三级结构的刚性和构象 （2, 4, 11, 12）。此外，AFP 被认为具有免疫抑制活性，调节细胞增殖和凋亡，启动细胞内信号传导，并有助于细胞入侵（4, 13）。AFP 的假定细胞表面受体已被描述过，AFP 被许多细胞类型所内化（14）。AFP 基因敲除小鼠是可以存活的，但雌性小鼠由于激素环境不足而无法正常排卵（15, 16）。

胎儿和母体AFP水平的改变与一些先天性异常有关，包括甲状腺功能减退、自身免疫性疾病和心脏缺陷（2, 17-19）。此外，AFP被广泛用作神经管缺陷和染色体异常的产前筛查标志物（2, 20）。母体血清AFP水平低与唐氏综合征的高发病率相关，而血清AFP较高水平与脊柱裂和无脑畸形相关（21-24）。出生后产生的AFP与几种病理有关。AFP是肝细胞癌的常用标志物，AFP水平升高与其他几种癌症相一致，包括肝母细胞瘤、生殖细胞瘤和某些胃癌（25-31）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠AFP抗体包被于微孔板上，样品和标准品中的小鼠AFP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AFP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。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血清样本；
- 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，应将样本用试剂稀释液（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、试剂盒的储存等。

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)	1947.6	504.8	124.5	2086.3	601.6	133.3
标准差	81.9	20.9	5.6	186.7	38.2	12.8
CV%	4.2	4.1	4.5	8.9	7.2	9.6

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AFP，测定其回收率。回收率范围在106.9-129.3%，平均回收率在118.3%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠AFP，测定其回收率。回收率范围在87.0-113.7%，平均回收率在101.3%。

C. 灵敏度

小鼠AFP的最低可测剂量（MDD）一般小于1.23 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NS0表达的高纯度重组小鼠AFP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AFP，然后用试剂稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数	平均值/期待值（%）	范围 (%)
1:2	93.6	89.2-95.9
1:4	92.9	85.1-103.7
1:8	91.9	83.0-105.5
1:16	93.7	85.7-110.2

F. 样本预值

血清样本 - 使用本试剂盒检测了5份小鼠血清样本中AFP的水平。5份样本的检测值在84.4 - 290.7 ng/mL之间，平均值为197.9 ng/mL。

G. 特异性

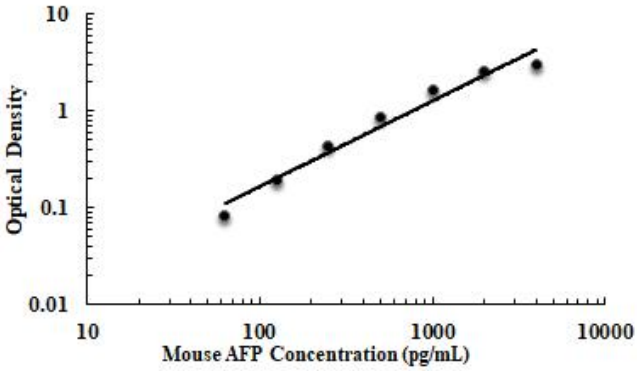
将以下因子配制成50 ng/mL的浓度来检测没有观察到明显的交叉反应或干扰。

Recombinant mouse	**Recombinant human**
Leptin	α-Fetoprotein
Prolactin	**Natural proteins**
Resistin	human α-Fetoprotein
Vitamin D BP	mouse serum albumin

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	0.080 0.082	0.081	-
62.5	0.162 0.168	0.165	0.084
125	0.264 0.286	0.275	0.194
250	0.493 0.532	0.513	0.432
500	0.900 0.989	0.945	0.864
1000	1.642 1.757	1.700	1.619
2000	2.517 2.653	2.585	2.504
4000	3.003 3.055	3.029	2.948

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse AFP Microplate	包被大鼠抗小鼠 AFP 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse AFP Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Mouse AFP Detection Antibody	生物素化的 AFP 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液（10×）	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 底物溶液	1 瓶
Stop Solution	终止液	1 瓶
Plate Sealers	封板膜	3 张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>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*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</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</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
- 振荡器（速度可调至500±50 rpm）
- 500 mL量筒

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。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。样本可能需要用试剂稀释液（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 x 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。样本可能需要用试剂稀释液（1×）稀释。

B. 样本准备工作

血清样本需要用试剂稀释液 (1×) 100倍稀释后进行检测，例如:10 µL样本加到90 µL试剂稀释液 (1×) 中，充分混匀，即10倍稀释。然后取10 µL 10倍稀释后的样本加到90 µL试剂稀释液 (1×) 中，充分混匀，即制备成100倍稀释的样本。

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浓缩洗涤液用去离子水或蒸馏水稀释配制成500 mL工作浓度的洗涤液。

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×）。

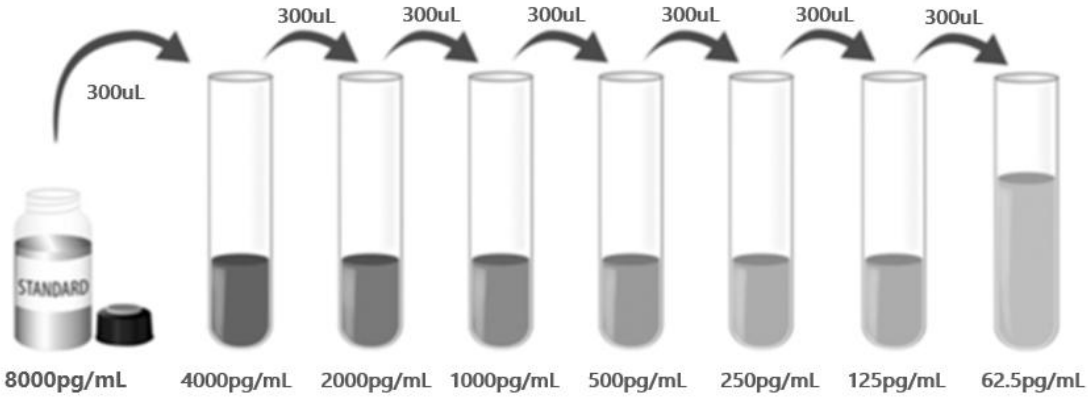
检测抗体：开盖前请瞬时离心。参考检测抗体瓶标签指示，用试剂稀释液（1×）将冻干粉进行重溶。再用试剂稀释液（1×）稀释至工作浓度200 ng/mL，至少在使用前15分钟准备。

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×）稀释至工作浓度链霉亲和素- HRP A（1×）。

AFP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8000 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试剂稀释液（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。4000 pg/mL管作标准曲线最高点，试剂稀释液（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，实验样本或者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±50rpm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。用封板膜封住反应孔，**室温500±50rpm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，**室温500±50rpm水平振荡孵育20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，**室温500±50rpm水平振荡孵育20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。孔内溶液颜色会从蓝色变为黄色；
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。如果没有使用双波长校正，结果准确度可能会受影响；
12. **计算结果：**将每个标准品和样品的校正吸光度值（OD450-OD540/OD570），复孔读数取平均值，然后减去平均零标准品OD值。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。另一种方法是，可以通过绘制标准品浓度做对数与相应OD值对数生成曲线，并通过回归分析确定最佳拟合线。通过样本的OD值，可从标准曲线上得到样本中小鼠AFP的浓度。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。

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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