PRODUCT INFORMATION & MANUAL

Mouse Angiopoietin-like 3 /ANGPT-L3 Valukine™ ELISA

Catalog Number: VAL632

For the quantitative determination of natural and recombinant mouse Angiopoietin-like 3/ANGPT-L3 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 202410.1

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I. BACKGROUND

Angiopoietin-like 3 (ANGPT-L3) is a secreted glycoprotein that plays an important role in fatty acid metabolism. It is one of several molecules with structural similarity to the angiopoietins, which also contain an N-terminal coiled coil domain and a C-terminal fibrinogen-like domain (1-4). Mouse ANGPT-L3 shares 22%-30% amino acid (aa) sequence identity with mouse ANGPT-L1, 2, 4, 6, and 7. It shares 77% aa sequence identity with human ANGPT-L3. ANGPT-L3 is expressed in the liver from early in development through adulthood (5, 6). It is found as 70 kDa, 50 kDa, and 32 kDa forms and can form weakly associated non-covalent multimers in vitro (6). ANGPT-L3 directly inhibits lipoprotein lipase (LPL) and endothelial lipase, enzymes responsible for hydrolyzing circulating triglycerides and HDL phospholipids (7, 8). This activity requires a putative heparin-binding motif which is N-terminal to the coiled coil domain (9).

ANGPT-L3 is proteolytically cleaved in the liver by proprotein convertases (10). Full length ANGPT-L3 circulates in the plasma as do the separated N- and C-terminal fragments which contain the coiled coil domain and fibrinogen-like domains, respectively (9). Cleavage serves to activate ANGPT-L3, as the released N-terminal fragment is more potent than full length ANGPT-L3 at increasing plasma triglycerides and inhibiting endothelial lipase (9, 10). ANGPT-L3 does not bind the angiopoietin receptors Tie-1 or Tie-2, but its fibrinogen-like domain interacts with integrin αVβ3 to induce endothelial cell adhesion, migration, and neovascularization (11). ANGPT-L3 also promotes the expansion of hematopoietic stem cells (12).

ANGPT-L3 promotes an increase in circulating triglyceride levels but does not alter VLDL or HDL secretion or uptake (7, 9, 13). ANGPT-L3 knockout mice are hypolipidemic and have elevated LPL activity (14). ANGPT-L3 expression in vivo is upregulated by liver X receptor (LXR) agonists and downregulated by insulin, leptin, and agonists of thyroid hormone receptor beta (TRβ) or peroxisome proliferator-activated receptor beta (PPARβ) (15-18). Dysregulated ANGPT-L3 expression and elevated plasma triglyceride levels are characteristic of some strains of obese and diabetic mice (7, 13, 16). In humans, serum ANGPT-L3 levels are positively correlated with serum HDL-Cholesterol and adiponectin levels as well as with arterial intima-media thickness, an indicator for the progression of atherosclerosis (19, 20).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse Angiopoietin-like 3 has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse Angiopoietin-like 3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse Angiopoietin-like 3 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 Angiopoietin-like 3 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 (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 forty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	190	383	1346	197	418	1449
Standard Deviation	10.9	23.6	79.1	17.4	27.1	90.5
CV%	5.7	6.2	5.9	8.8	6.5	6.2

B. RECOVERY

The recovery of mouse Angiopoietin-like 3 spiked to levels throughout the range of the assay was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture media (n=4)	103	93-120

C. SENSITIVITY

Fifty-two assays were evaluated and the minimum detectable dose (MDD) of mouse Angiopoietin-like 3 ranged from 1.64-9.62 pg/mL. The mean MDD was 4.29 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 Angiopoietin-like 3 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 Angiopoietin-like 3 and diluted with Calibrator Diluent

(1×) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=2)	Mouse serum* (n=4)	Mouse EDTA plasma* (n=4)	Mouse heparin plasma* (n=4)
1:2	Average % of Expected	101	98	98	96
	Range (%)	100-101	96-99	93-102	91-99
1:4	Average % of Expected	103	98	98	95
	Range (%)	100-106	97-100	90-105	90-101
1:8	Average % of Expected	103	99	96	92
	Range (%)	97-110	96-102	88-103	87-99
1:16	Average % of Expected	91	97	96	92
	Range (%)	91-91	93-99	88-105	85-96

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

F. SAMPLE VALUES

Mouse serum/plasma - Samples were evaluated for detectable levels of mouse Angiopoietin-like 3 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Mouse serum (n=20)	244	115-435	78.7
Mouse EDTA plasma (n=20)	311	137-486	89.6
Mouse heparin plasma (n=20)	234	139-343	52.0

Cell Culture Supernates:

Mouse liver tissue from one mouse was cut into 1-2 mm pieces and cultured in 100 mL of RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 2 days. Cells were unstimulated or stimulated with 1.0 mg/mL of lipopolysaccharide for 2 days. Aliquots of the cell culture supernates were removed and assayed for mouse Angiopoietin-like 3.

Mouse heart tissue from two mice was cut into 1-2 mm pieces and cultured in 100 mL of RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 1 day. Cells were unstimulated or stimulated with 1.0 mg/mL of lipopolysaccharide for 1 day. Aliquots of the cell culture supernates were removed and assayed for mouse Angiopoietin-like 3.

Mouse lung tissue from three mice was cut into 1-2 mm pieces and cultured in 100 mL of RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 1 day. An aliquot of the cell culture supernate was removed and assayed for mouse Angiopoietin-like 3.

Tissue Type	Observed Levels (pg/mL)
Liver, unstimulated	918
Liver, stimulated	1045
Heart, unstimulated	152
Heart, stimulated	131
Lung	232

G. SPECIFICITY

This assay recognizes natural and recombinant mouse Angiopoietin-like 3.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent (1×) and

assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse Angiopoietin-like 3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:	**Recombinant human:**
Angiopoietin-3	Angiopoietin-like 3 (aa 17-460)
Angiopoietin-like 2	Angiopoietin-like 3 (aa 17-220)
Angiopoietin-like 4	LPL (aa 28-154)
Angiopoietin-like7	

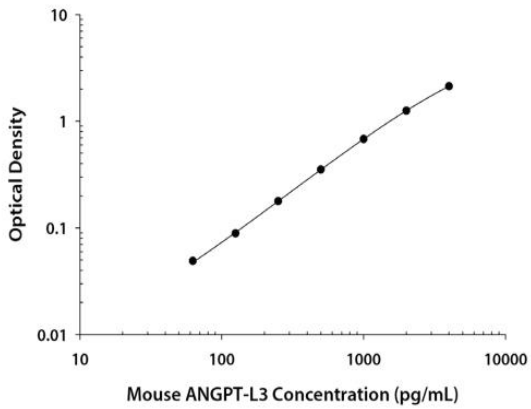
Rat serum was not detectable in this assay.

This assay detects full-length and 27 kDa N-terminal cleaved mouse Angiopoietin-like 3 but does not detect the 38 kDa C-terminal cleavage fragment.

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	0.014 0.018	0.016	—
62.5	0.062 0.067	0.065	0.049
125	0.102 0.108	0.105	0.089
250	0.190 0.197	0.194	0.178
500	0.357 0.380	0.369	0.353
1000	0.691 0.702	0.697	0.681
2000	1.230 1.313	1.272	1.256
4000	2.067 2.220	2.144	2.128

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse Angiopoietin-like 3 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse Angiopoietin-like 3.	1 plate
Mouse Angiopoietin-like 3 Conjugate	An antibody specific for mouse Angiopoietin-like 3 conjugated to horseradish peroxidase.	1 vial
Mouse Angiopoietin-like 3 Standard	Recombinant mouse Angiopoietin-like 3 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Mouse Angiopoietin-like 3 Control	Recombinant mouse Angiopoietin-like 3 in a buffered protein base; lyophilized. The assay value of the control should be within the range specified on the label.	2 vials
Assay Diluent RD1-21	A buffered protein base.	1 vial
Calibrator Diluent Concentrate (4×)/ RD5-26	A 4× concentrated 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 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="5">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Assay Diluent RD1-21</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td rowspan="2">Use a new standard and control for each assay. Discard after use.</td></tr>
<tr><td>Control</td></tr>
<tr><td>Calibrator Diluent Concentrate (4×)/ RD5-26</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>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.
- 100 mL and 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12"orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Polypropylene test tubes for dilution of standards 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 (1×).

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Plasma - Collect plasma using EDTA or 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 (1×).

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

Do not use icteric samples.

B. SAMPLE PREPARATION

Mouse serum and plasma samples recommend a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent (1×). followed by 70 µL of the diluted sample + 70 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse Angiopoietin-like 3 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×).

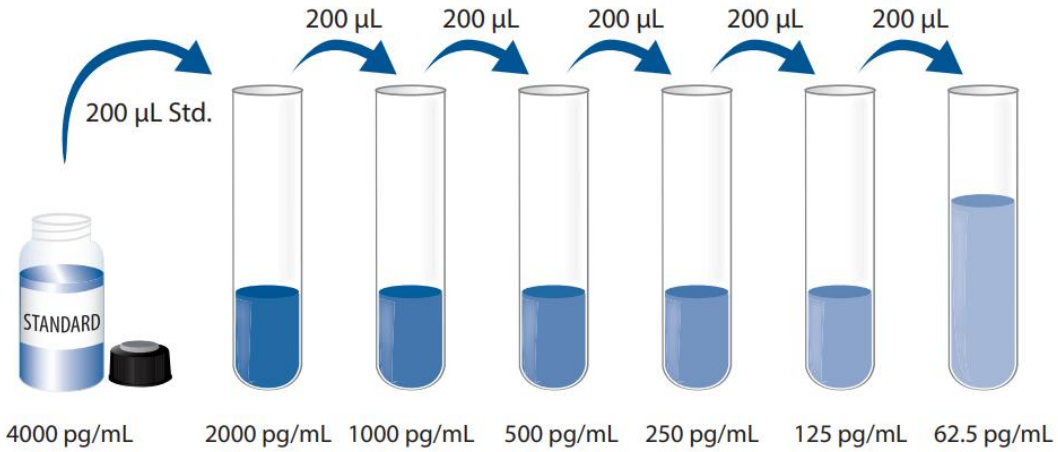
Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

Mouse Angiopoietin-like 3 Standard- Refer to the vial label for the reconstitution volume* Reconstitute the Mouse Angiopoietin-like 3 Standard with Calibrator Diluent (1×). 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 (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (4000 pg/mL). 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

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, working 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 RD1-21 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 Angiopoietin-like 3 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 on the benchtop. 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 Angiopoietin-like 3 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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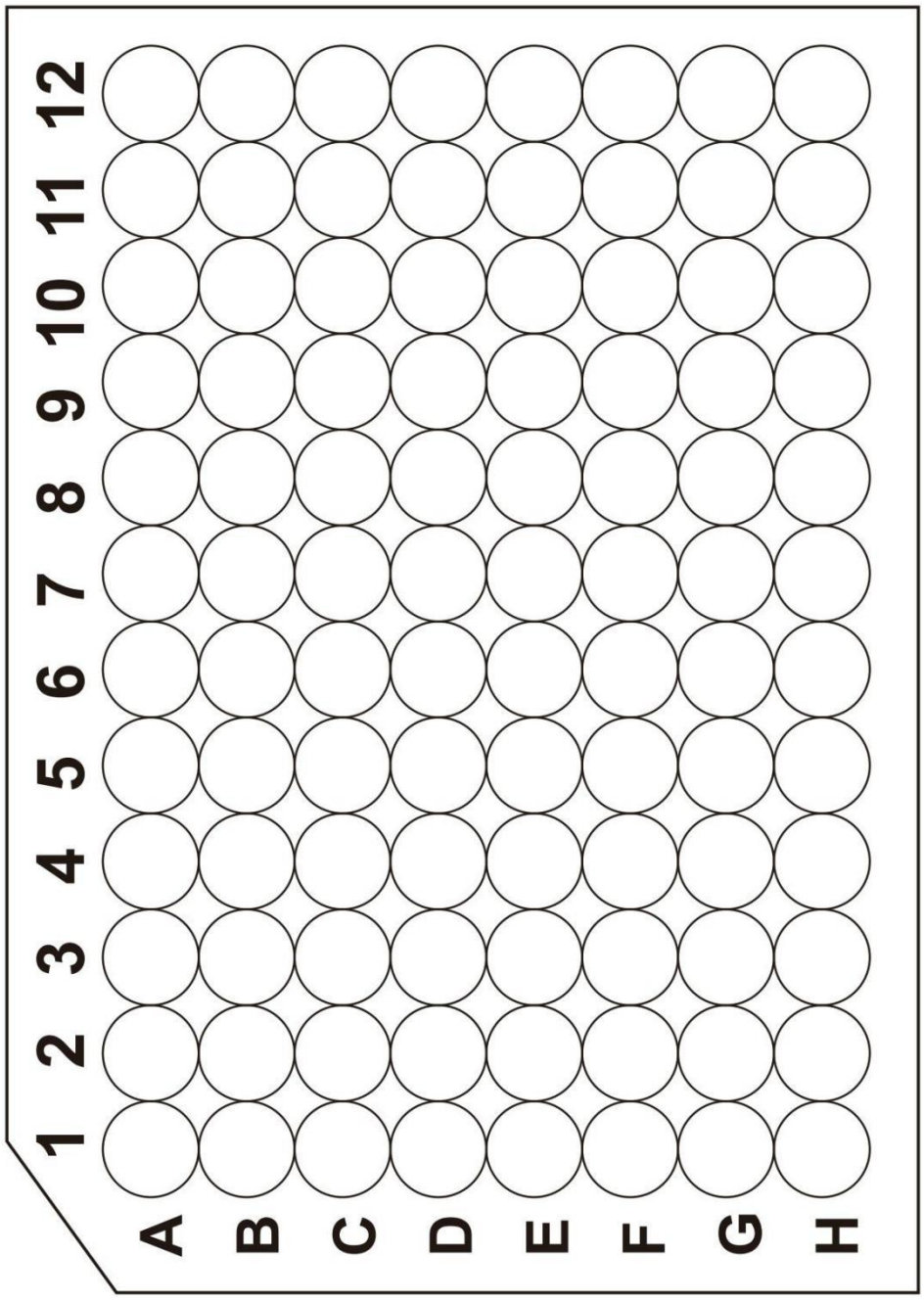
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 Angiopoietin-like 3 /ANGPT-L3 Valukine™ ELISA 试剂盒

目录号：VAL632

适用于定量检测天然和重组小鼠 Angiopoietin-like 3/ANGPT-L3 的浓度

科研专用，不可用于临床诊断

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版本号 202410.1

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I. 背景

血管生成素样 3（Angiopoietin-like 3, ANGPT-L3）是一种分泌型糖蛋白，在脂肪酸代谢中发挥着重要作用。它是与血管生成素具有结构相似性的几个分子之一，血管生成素也包含 N 末端盘绕的卷曲螺旋结构域和 C 末端纤维蛋白原样结构域（1-4）。小鼠 ANGPT-L3 与小鼠 ANGPT-L1、2、4 有 22%-30%的氨基酸（amino acid, aa）序列相同性。它与人 ANGPT-L3 有 77％的氨基酸序列相同性。ANGPT-L3 从发育初期到成年期在肝脏中表达（5, 6）。它有 70 kDa、50 kDa 和 32 kDa 三种形态，在体外可形成弱相关的非共价多聚体（6）。ANGPT-L3 可直接抑制脂蛋白脂肪酶（lipoprotein lipase, LPL）和内皮脂肪酶，这些酶负责水解循环中的甘油三酯和 HDL 磷脂（7, 8）。这种活性需要一个假定的肝素结合基团，该基团位于卷曲螺旋结构域的 N 端（9）。

ANGPT-L3 通过前蛋白转化酶在肝脏种蛋白分解裂解（10）。全长 ANGPT-L3 在血浆中循环，分离出的 N 端和 C 端片段分别含有卷曲螺旋结构域和纤维蛋白原样结构域（9）。裂解可激活 ANGPT-L3，因为释放的 N 端片段在增加血浆甘油三酯和抑制内皮脂肪酶时比全长 ANGPT-L3 更有效（9, 10）。ANGPT-L3 不与血管生成素受体 Tie-1 或 Tie-2 结合，但其纤维蛋白原样结构域与整合素αVβ3 相互作用，诱导内皮细胞粘附、迁移和新生血管形成（11）。ANGPT-L3 还能促进造血干细胞的扩增（12）。

ANGPT-L3 可促进循环中甘油三酯水平的增加，但不会改 VLDL 或 HDL 的分泌或吸收（7, 9, 13）。ANGPT-L3 基因敲除小鼠血脂偏低，LPL 活性升高（14）。肝 X 受体（liver X receptor, LXR）激动剂上调体内 ANGPT-L3 的表达，而受胰岛素、瘦素和甲状腺激素受体 beta（thyroid hormone receptor beta, TRβ）激动剂或过氧化物酶体增殖激活受体 beta（peroxisome proliferator-activated receptor beta, PPARβ）激动剂下调体内 ANGPT-L3 的表达（15-18）。ANGPT-L3 表达失调和血浆甘油三酯水平升高是肥胖和糖尿病小鼠某些品系的特征（7, 13, 16）。在人体中，血清 ANGPT-L3 水平与血清 HDL-胆固醇和脂联素水平以及动脉内膜厚度呈正相关，而动脉内膜厚度是动脉粥样硬化进展的指标（19, 20）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠Angiopoietin-like 3抗体包被于微孔板上，样品,质控品和标准品中的小鼠Angiopoietin-like 3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Angiopoietin-like 3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，小鼠血浆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，在不同板间分别检测40次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	190	383	1346	197	418	1449
标准差	10.9	23.6	79.1	17.4	27.1	90.5
CV%	5.7	6.2	5.9	8.8	6.5	6.2

B. 回收率

在样本中掺入检测范围内不同水平的小鼠Angiopoietin-like 3，测定其回收率。

样本类型	平均回收率%	范围 (%)
细胞培养基 (n=4)	103	93-120

C. 灵敏度

52次检测结果表明，小鼠Angiopoietin-like 3的最低可测剂量（MDD）范围为1.64- 9.62 pg/mL。平均MDD为4.29 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Angiopoietin-like 3校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠Angiopoietin-like 3，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		细胞培养基 (n=2)	小鼠血清* (n=4)	小鼠EDTA血浆* (n=4)	小鼠肝素血浆* (n=4)
1:2	平均值/期待值（%）	101	98	98	96
	范围（%）	100-101	96-99	93-102	91-99
1:4	平均值/期待值（%）	103	98	98	95
	范围（%）	100-106	97-100	90-105	90-101
1:8	平均值/期待值（%）	103	99	96	92
	范围（%）	97-110	96-102	88-103	87-99
1:16	平均值/期待值（%）	91	97	96	92
	范围（%）	91-91	93-99	88-105	85-96

*样品在测定前按照样品制备部分的指示进行稀释

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Angiopoietin-like 3的可检测水平的评估。

样本类型	平均值 (ng/mL)	范围 (ng/mL)	标准差(ng/mL)
小鼠血清样本 (n=20)	244	115-435	78.7
小鼠EDTA血浆样本 (n=20)	311	137-486	89.6
小鼠肝素血浆样本 (n=20)	234	139-343	52.0

细胞上清样本:

将1只小鼠肝组织切成1-2 mm块，培养在含有10%胎牛血清、50 µM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素的100 mL RPMI培养基中，培养

2天。1.0 mg/mL脂多糖刺激细胞2天或不刺激细胞。取出等量的细胞培养上清，检测小鼠Angiopoietin-like 3的含量。

将2只小鼠心脏组织切成1-2 mm块，培养在含有10%胎牛血清、50 μM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 μg/mL硫酸链霉素的100 mL RPMI培养基中，培养1天。1.0 mg/mL脂多糖刺激细胞1天或不刺激细胞。取出等量的细胞培养上清，检测小鼠Angiopoietin-like 3的含量。

将3只小鼠肺组织切成1-2 mm块，培养在含有10%胎牛血清、50 μM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 μg/mL硫酸链霉素的100 mL RPMI培养基中，培养1天。取出等量的细胞培养上清，检测小鼠Angiopoietin-like 3的含量。

组织类型	**检测值 (pg/mL)**
肝脏，不刺激	918
肝脏，刺激	1045
心脏，不刺激	152
心脏,刺激	131
肺	232

G. 特异性

此ELISA法可检测天然及重组小鼠Angiopoietin-like 3。

将以下因子用标准品稀释液（1×）配制成50 ng/mL的浓度来检测与小鼠Angiopoietin-like 3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Angiopoietin-like 3对照品中，来检测对小鼠Angiopoietin-like 3的干扰。没有观察到明显的交叉反应或干扰。

Recombinant mouse:	**Recombinant human:**
Angiopoietin-3	Angiopoietin-like 3 (aa 17-460)
Angiopoietin-like 2	Angiopoietin-like 3 (aa 17-220)
Angiopoietin-like 4	LPL (aa 28-154)
Angiopoietin-like 7	

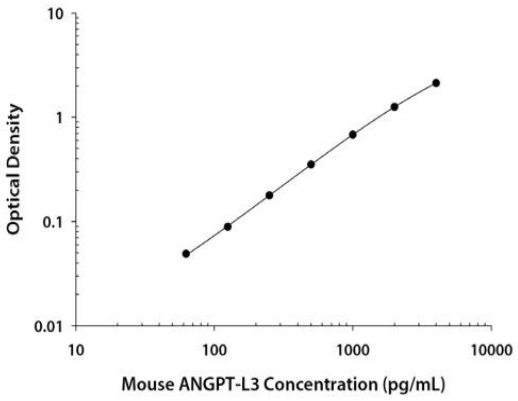
本试剂盒不能检测大鼠血清样本。

本试剂盒能检测全长和27 kDa的N端剪切小鼠Angiopoietin-like 3，但不检测38 kDa C端剪切片段。

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	0.014 0.018	0.016	—
62.5	0.062 0.067	0.065	0.049
125	0.102 0.108	0.105	0.089
250	0.190 0.197	0.194	0.178
500	0.357 0.380	0.369	0.353
1000	0.691 0.702	0.697	0.681
2000	1.230 1.313	1.272	1.256
4000	2.067 2.220	2.144	2.128

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse Angiopoietin-like 3 Microplate	包被抗小鼠Angiopoietin-like 3抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Mouse Angiopoietin-like 3 Conjugate	酶标检测抗小鼠Angiopoietin-like 3抗体	1瓶
Mouse Angiopoietin-like 3 Standard	小鼠Angiopoietin-like 3标准品（冻干），参考瓶身标签进行重溶	2瓶
Mouse Angiopoietin-like 3 Control	小鼠Angiopoietin-like 3质控品（冻干），质控品的测定值应在标签上规定的范围内	2瓶
Assay Diluent RD1-21	检测液	1瓶
Calibrator Diluent Concentrate (4×)/ RD5-26	浓缩标准品稀释液（4×）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="9">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="5">2-8℃储存，最多30天*</td></tr>
<tr><td>检测液RD1-21</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</td><td rowspan="2">每次使用新鲜的标准品和质控品，使用后丢弃</td></tr>
<tr><td>质控品</td></tr>
<tr><td>浓缩标准品稀释液（4×）/RD5-26</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>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
- 100mL和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聚丙烯管

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清：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（1×）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℃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EDTA或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℃储存备用。避免反复冻融。样本可能需要用稀释液（1×）稀释。

注意：*本试剂盒对柠檬酸钠血浆尚未被验证。*

不使用黄疸样本。

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（1×）100倍稀释后进行检测，即10 µL血清+490 µL标准品稀释液（1×）。然后70 µL稀释后样品+70µL标准品稀释液（1×），即是100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Angiopoietin-like 3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×）。

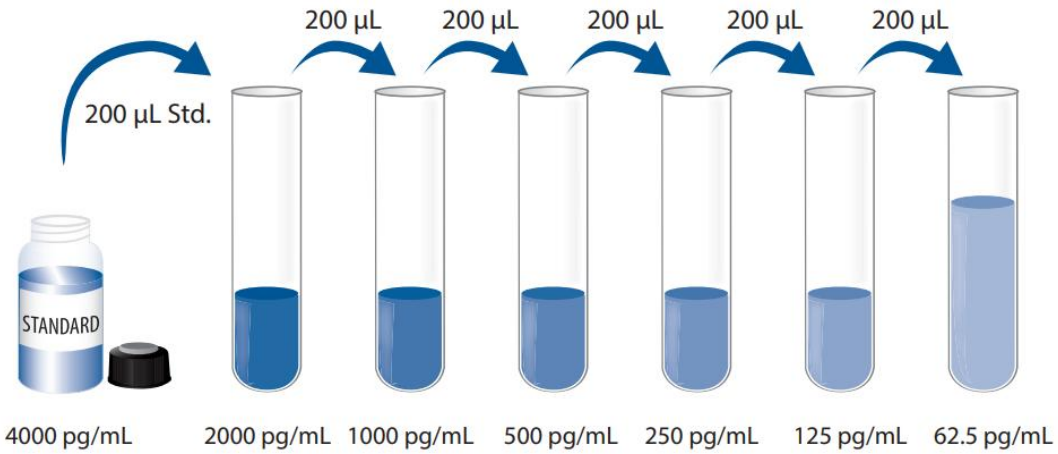
标准品稀释液（1×）：使用蒸馏水或去离子水稀释配制成标准品稀释液（1×）。

小鼠Angiopoietin-like 3标准品：重溶体积请参考瓶身标签*，用标准品稀释液（1×）重溶小鼠Angiopoietin-like 3标准品。不得使用其他试剂。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。

每个稀释管中加入200 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。没有稀释的标准品母液可用作标准曲线最高点

（4000 pg/mL），标准品稀释液（1×）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RD1-21。
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）转速：500±50 rpm上，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Angiopoietin-like 3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）转速：500±50 rpm上，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Angiopoietin-like 3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