叶酸/维生素 B9(FA/VB9)酶联免疫吸附测定试剂盒

使用说明书

产品编号：D751003

包装规格：48 TESTS / 96 TESTS

声明：

使用前仔细阅读本说明书。只能用于研究用途，不得用于医学诊断。

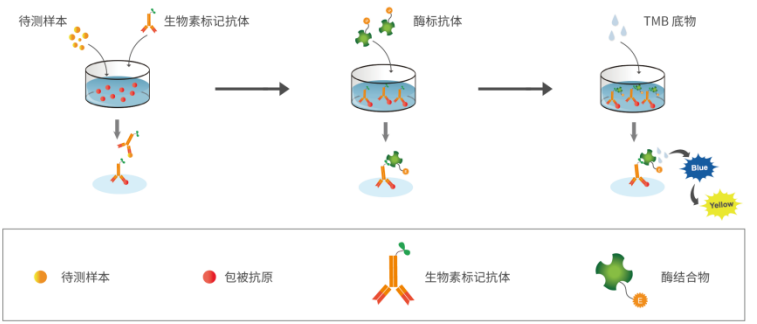
用途

用于血清、血浆或其他相关生物液体中叶酸/维生素 B9 的测定。

工作原理

本试剂盒采用的是竞争酶联免疫吸附检测技术（ELISA）。测定样品中叶酸/维生素 B9 水平。向预先包被了叶酸/维生素 B9 抗原的酶标孔中，加入标准品和样本，温育后，加入生物素标记的抗叶酸/维生素 B9 抗体。再与 HRP 标记的链霉亲和素结合，形成免疫复合物，再经过温育和洗涤，去除未结合的酶，然后加入显色底物 TMB，产生蓝色，并在酸的作用下转化成最终的黄色。最后，在 450 nm 处测定反应孔样品吸光度（OD）值，样本中的叶酸/维生素 B9 浓度与 OD 值成反比，通过绘制标准曲线计算出样本中叶酸/维生素 B9 的浓度。

原理图：



试剂盒组成

试剂盒组成	48 孔配置	96 孔配置	保存
说明书	1 份	1 份	
封板膜	5 片	5 片	
预包被酶标板	8 孔 X 6 条	8 孔 X 12 条	-20°C
标准品	1 瓶	2 瓶	-20°C
标准品/样本稀释液 SD1	20 mL X 1 瓶	20 mL X 1 瓶	2-8°C
浓缩生物素标记叶酸/维生素 B9 抗体（100X）	60 µl	120 µl	-20°C
生物素标记抗体稀释液 SD2	14 mL X 1 瓶	14 mL X 1 瓶	2-8°C
浓缩 HRP 标记链霉亲和素（100X）	60 µl	120 µl	-20°C （避光）

HRP 标记链霉亲和素稀释液 SD3	14 mL X 1 瓶	14 mL X 1 瓶	2-8°C
显色剂	10 mL X 1 瓶	10 mL X 1 瓶	2-8°C （避光）
终止液	10 mL X 1 瓶	10 mL X 1 瓶	2-8°C
浓缩洗涤液（25×）	30 mL X 1 瓶	30 mL X 1 瓶	2-8°C

需要而未提供的试剂和器材

1. 37°C 恒温箱
2. 酶标仪（450 nm 波长滤光片）
3. 精密移液器及一次性吸头
4. 去离子水或蒸馏水
5. 一次性试管
6. 洗板机或洗瓶，吸水纸

注意事项

1. 试剂盒应在有效期内使用，请不要使用过期的试剂。不同批次的试剂盒组分不能混用。
2. 试剂盒未使用时应按各组分标签温度分开保存。
3. 试剂盒使用前请在室温恢复 20 min，且充分混匀试剂盒里的各种成份及制备的样品。
4. 在试验中标准品和样本建议作复孔检测，且加入试剂的顺序应保持一致。
5. 为避免交叉污染，请在试验中使用 1 次性试管，枪头，封板膜及洁净塑料容器。.
6. 浓缩生物素标记抗体和浓缩 HRP 标记链霉亲和素的体积较少，在运输过程中微量液体会沾到管壁及瓶盖上，使用前请离心处理（5-10 s 即可），使管壁上的液体集中在管底部，取用时，请用移液器小心吹打几次。
7. 为保证结果准确，每次检测均需做标准曲线。请勿重复使用已稀释过的标准品、浓缩生物素标

记抗体、浓缩 HRP 标记链霉亲和素。

8. 试剂盒中的终止液为酸性溶液，操作人员在使用时请带上手套并注意防护；在操作过程中也要避免试剂接触皮肤和眼睛，如果不慎接触，请用大量清水清洗；检测血液样本及其它体液样本时，请按国家生物实验室安全防护有关管理规定执行。

样本收集及储存

1. 细胞培养上清：

   将细胞培养基移至无菌离心管，在 4°C 条件下 1000 X *g* 离心 20 min，除去杂质及细胞碎片。取上清检测。

2. 血清样本：

   室温下血液自然凝固后，在 4°C 条件下 1000 X *g* 离心 20 min，取上清即可检测。

3. 血浆样本：

   将全血收集到含抗血凝剂的管中，抗凝剂推荐使用 EDTA 钠盐，样品采集后 30 min 内于 1000 X *g* 离心 15 min，取上清即可检测。避免使用溶血，高血脂样品。

4. 组织匀浆：用预冷的 PBS （0.01 M, pH=7.4）冲洗组织，去除残留血液，称重后将组织剪碎。将剪碎的组织与对应体积的 PBS （一般按 1:9 的重量体积比，比如 1 g 的组织样品对应 9 mL 的 PBS，具体体积可根据实验需要适当调整，并做好记录。推荐在 PBS 中加入蛋白酶抑制剂）加入玻璃匀浆器中，在冰上充分研磨。为了进一步裂解组织细胞，可以对匀浆液进行超声破碎。最后将匀浆液 5000 X *g* 离心 5-10 min，取上清检测。

5. 细胞提取液：贴壁细胞用冷的 PBS 轻轻清洗，然后用胰蛋白酶消化，1000 X *g* 离心 5 min 后收集细胞；悬浮细胞可直接离心收集。收集的细胞用冷的 PBS 洗涤 3 次。每 1×10^6 个细胞中加入 150-200 μl PBS 重悬并通过反复冻融使细胞破碎(若含量很低可减少 PBS 的体积)。将提取液于 1500 X *g* 离心 10 min，取上清检测。

※注意：

1. 样品收集后若在 1 周内进行检测的可保存于 4°C，若不能及时检测，请按一次使用量分装，冻存于 -20°C (1 个月内检测)，或 -80°C (3 个月内检测)，避免反复冻融。

2. 试剂盒检测范围不等同于样本的浓度范围，如果您的样品中检测物浓度高于标准品最高值，请根据实际情况，做适当倍数稀释 (建议查阅文献后先做预实验，以确定稀释倍数)。

3. 若所检样本不在说明书所列样本之中，建议做预实验验证其检测有效性。

4. 若使用化学裂解液制备组织匀浆或细胞提取液，由于引入某些化学物质会导致 ELISA 测值出现偏差。

5. 某些重组蛋白可能与试剂盒中捕获或检测抗体不匹配而出现不能检测的情况。

试剂准备

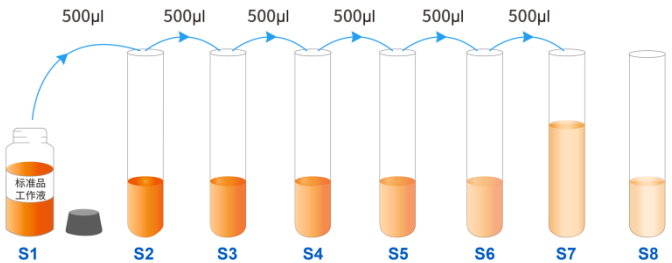
1. 试剂回温：在实验前 20 min 将试剂盒、待测样本放置于室温下。读数前 15 min 打开酶标仪预热。

2. 配制洗涤液：预先计算好稀释后的洗涤液使用体积，然后用双蒸水或去离子水将 25 倍浓缩洗涤液稀释成 1 倍应用液。

   提示：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象，可用 40°C 水浴微加热使结晶完全溶解后再配制洗涤液。当日使用。

3. 标准品梯度稀释：标准品于 10000 X *g* 离心 1 min，加入标准品&样品稀释液 1.0 mL 至冻干标准品中，旋紧管盖，静置 10 min，上下颠倒数次，待其充分溶解后，轻轻混匀，配成 100 ng/mL 的标准品工作液。然后根据需要进行倍比稀释。建议配制成以下浓度：100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0 ng/mL。倍比稀释方法：取 7 支 EP 管，每管中加入 500 µl 标准品&样品稀释液，从 100 ng/mL 的标准品工作液中吸取 500 µl 到其中一支 EP 管中混匀配成 50 ng/mL 的标准品工作液，按此步骤往后依次吸取混匀。具体稀释如下图 S1-S8。

   提示：最后一管直接作为空白孔，不需要再从倒数第二管中吸取液体。



4. 生物素标记抗体工作液：预先计算好试验所需用量(以50 μl/孔计算)，用稀释液SD2将100倍生物素标记抗体浓缩液稀释成1倍工作液（稀释前充分混匀），请在30 min内加入到反应孔中。

5. HRP 标记链霉亲和素：按每次试验所需用量配制(以100 μl/孔计算)，用稀释液SD3将100倍浓缩 HRP 标记链霉亲和素稀释成1倍应用工作液（稀释前离心），请在30 min内使用。

6. 洗涤方法：

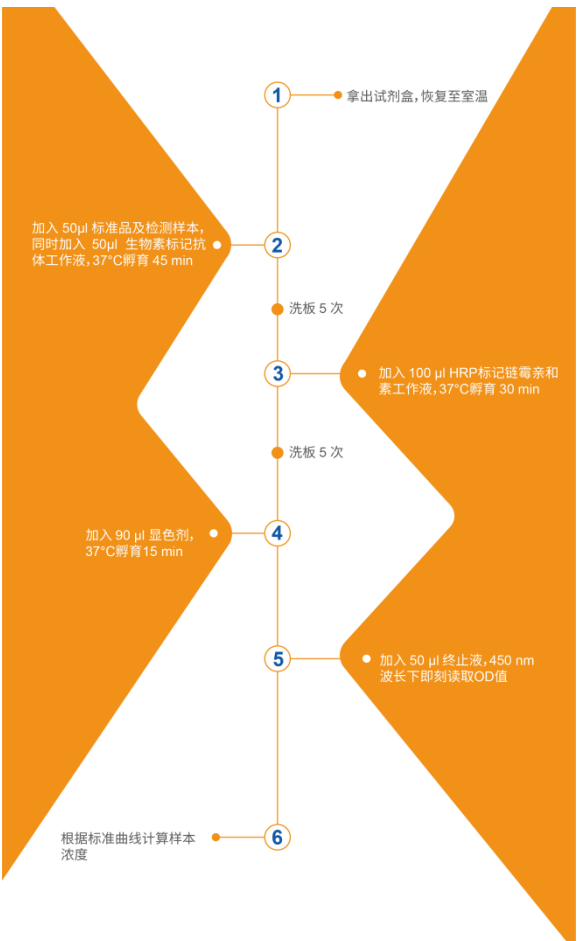
   - 自动洗板：甩尽酶标板孔中液体，在厚迭吸水纸上拍干，注入洗涤液为 350 μl/孔,注入与吸出间隔为 30 s，洗板 5 次。
   - 手工洗板：甩尽酶标板孔中液体，在厚迭吸水纸上拍干，用洗瓶加入洗涤液 350 μl/孔，静止 1-2 min 后甩净酶标板孔中液体，在厚迭的吸水纸上拍干，洗板 5 次。

操作程序

1. 预先计算好所需的板条数，实验前 30 min，拿出试剂盒，恢复至室温。
2. 每个反应孔中加入 50 μl 标准品工作液及检测样本(若样本浓度高于检测范围，需用标准品&样本稀释液稀释后取样)，标准品需做复孔。每个反应孔中立即加入 50 μl 生物素标记叶酸/维生素 B9 抗体工作液，封板后于 37°C 孵箱孵育 45 min。

   提示：加样时将样品加于酶标板底部，尽量不触及孔壁，轻轻晃动混匀，避免产生气泡。加样时间控制在 10 min 内。
3. 洗涤：弃去液体，甩干，每个反应孔中加入 350 μl 洗涤液，浸泡 1-2 min，甩干洗涤液。重复 4 次。
4. 每个反应孔中加入 100 μl HRP 标记链霉亲和素工作液，封板后于 37°C 孵箱孵育 30 min。
5. 洗涤：每个反应孔中加入 300 μl 洗涤液，间隔 30 s，甩干洗涤液。重复 4 次。
6. 每个反应孔中加入 90 μl 显色剂（避光），封板后于 37°C 避光显色 15 min 左右。
7. 每个反应孔中加入 50 μl 终止液，即刻用酶标仪 450 nm 波长下测量 OD 值（5 min 内）。
8. 用酶标仪 450 nm 波长测定 OD 值。
9. 以标准品浓度为横坐标，吸光度 OD 值为纵坐标，用“四参数 logistics 模型”绘制标准曲线。
10. 若样本 OD 值高于标准曲线上限，应做适当稀释后重新检测，计算浓度时再乘以稀释倍数。

操作程序图


1
拿出试剂盒，恢复至室温
加入 50μl 标准品及检测样本，同时加入 50μl 生物素标记抗体工作液，37°C孵育 45 min
2
洗板 5 次
3
加入 100 μl HRP标记链霉亲和素工作液，37°C孵育 30 min
洗板 5 次
加入 90 μl 显色剂，37°C孵育15 min
4
5
加入 50 μl 终止液，450 nm 波长下即刻读取OD值
根据标准曲线计算样本浓度
6

灵敏度

最低可检测浓度达 0.94 ng/mL。

检测范围

1.56-100 ng/mL

特异性

可检测样本中的 叶酸/维生素 B9，且与其类似物无明显交叉反应。

重复性

三种不同浓度的样本在同一块板子上分别检测 20 次，以及在不同的板子上分别检测 20 次，板内和板间变异系数均小于 10%。

重复性	板内差			板间差		
样本	低浓度	中浓度	高浓度	低浓度	中浓度	高浓度
检测次数	20	20	20	20	20	20
平均浓度（ng/mL）	4.59	9.64	47.95	4.96	9.5	47.2
标准差	0.29	0.55	1.78	0.32	0.43	1.53
变异系数（%）	6.32%	5.71%	3.71%	6.45%	4.53%	3.24%

回收率

分别往稀释不同倍数的 5 个样本中加入已知浓度的目标蛋白，做回收实验，得出回收率范围和平均回收率。

稀释倍数	血清 (n=5)	EDTA 血浆 (n=5)	细胞培养上清(n=5)	回收率
原液	**93-107**	**88-102**	**91-107**	回收率范围 (%)
	98	**93**	**99**	平均回收率 (%)
1:2	**91-107**	**91-103**	**93-110**	回收率范围 (%)
	98	**97**	**100**	平均回收率 (%)
1:4	**93-106**	**91-102**	**95-108**	回收率范围 (%)
	98	**97**	**97**	平均回收率 (%)
1:8	**91-106**	**90-102**	**95-111**	回收率范围 (%)
	98	**96**	**102**	平均回收率 (%)

1:16	**88-101**	**90-102**	**93-106**	回收率范围 (%)
	95	**94**	**100**	平均回收率 (%)

常见问题

问题	**可能原因**	**解决方法**
标准曲线梯度差	吸液或加液不准	检查移液器及吸头
	标准品稀释不正确	溶解标准品时稍微旋转瓶身，轻轻混匀使粉末完全溶解
	洗涤不完全	保证洗涤时间和洗涤次数及每孔的加液量
显色很弱或无色	孵育时间太短	保证充足的孵育时间
	实验温度不正确	使用推荐的实验温度
	试剂体积不够或漏加	检查吸液及加液过程，保证所有试剂按顺序足量添加
	稀释不正确	检查吸液及加液过程，保证所有试剂按顺序足量添加
	酶标记物失活或底物失效	混合酶结合物和底物，通过迅速显色来检查判断
读数数值低	酶标仪设置不正确	在酶标仪上检查波长及滤光片设置
		提前打开酶标仪预热
变异系数大	加液不正确	检查加液情况

背景值高	检测抗体的工作浓度过高	使用推荐的稀释倍数
	酶标板洗涤不完全	保证每步清洗完全；如果用自动洗板机，请检查所有的出口是否有堵塞；是否使用试剂盒配备的洗涤液
	洗液有污染	配制新鲜的洗液
灵敏度低	ELISA 试剂盒保存不当	按说明书要求保存相关试剂
	读数前未终止	OD 读数前应在每孔中加入终止液

FA/VB9 (Folic Acid/Vitamin B9) ELISA Kit

Instruction

Cat. No.：D751003

Package：48 TESTS/96 TESTS

Instruction

Please read the instructions carefully before the experiment. This kit is only for scientific research, not for use in diagnostic procedures.

Purpose

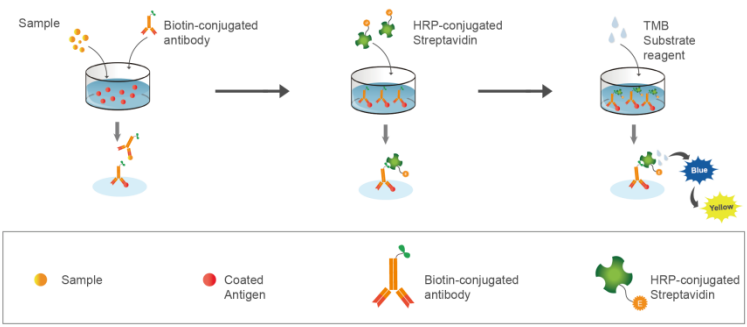
For the determination of Universal FA/VB9(Folic Acid/Vitamin B9) concentrations in serum, plasma and other related biological fluids.

Principle

This assay employs the competitive enzyme immunoassay technique. Determine the level of Universal FA/VB9(Folic Acid/Vitamin B9) in the sample. Add standard and sample to the microplate that have been pre-coated with Universal FA/VB9(Folic Acid/Vitamin B9) antigen. After incubation, add Biotin-conjugated anti-Universal FA/VB9(Folic Acid/Vitamin B9) antibody. It is then combined with HRP-conjugated streptavidin to form an immune complex, then incubated and washed to remove unbound enzyme, and then added to the chromogenic substrate TMB to produce a blue color, and

converted to the final yellow under the action of acid. Finally, the absorbance (OD) value was measured at 450 nm. The concentration of Universal FA/VB9(Folic Acid/Vitamin B9) in the sample was proportional to the OD value. The concentration of Universal FA/VB9(Folic Acid/Vitamin B9) in the sample can be calculated by drawing a standard curve.

Schematic diagram



Kit Components

Kit Components	48 determinations	96 determinations	storage
Instruction manual	1	1	
Closure plate membrane	5 pieces	5 pieces	
Pre-coated micro well plates	8 wells X 6 strips	8 wells X 12 strips	-20°C
Standard	1 vial	2 vials	-20°C

Standard/Sample diluent (SD1)	20 mL X 1 bottle	20 mL X 1 bottle	2-8°C
Concentrated Biotin-conjugated FA/VB9(Folic Acid/Vitamin B9) antibody (100X)	60 µl	120 µl	-20°C
Biotin-conjugated antibody diluent (SD2)	14 mL X 1 bottle	14 mL X 1 bottle	2-8°C
Concentrated HRP-conjugated Streptavidin (100X)	60 µl	120 µl	-20°C (Hiding from light)
HRP-conjugated Streptavidin diluent (SD3)	14 mL X 1 bottle	14 mL X 1 bottle	2-8°C
Substrate reagent	10 mL X 1 bottle	10 mL X 1 bottle	2-8°C (Hiding from light)
Stop Solution	10 mL X 1 bottle	10 mL X 1 bottle	2-8°C
Concentrated wash solution(25X)	30 mL X 1 bottle	30 mL X 1 bottle	2-8°C

Materials required but not supplied

1. 37°C incubator.
2. Microplate reader (450 nm wavelength filter).
3. Precision pipettes and Disposable pipette tips.
4. Deionized or distilled water.
5. Disposable Test tube.
6. Automated microplate washer or wash bottle, absorbent paper.

Important notes

1. This ELISA Kit should not be used beyond the expiration date on the kit label. Kit components of different batches cannot be used interchangeably.
2. This kit should be stored at the label temperature of each component when unused.
3. Recover the kit at room temperature for 20 min before use, and thoroughly mix the various components and samples prepared in the kit.
4. In the test, it is recommended that all standards and samples be assayed in duplicate. And the order of adding reagents should be consistent.
5. To avoid cross-contamination, please use the disposable test tube, the tip, the closure plate membrane and the clean plastic container in the test.
6. Concentrated Biotin-conjugated antibody and concentrated HRP-conjugated streptavidin have a small volume. During transport, trace liquid will adhere to the tube wall and cap. Please centrifuge before use (5-10 s), then the liquid on the tube wall is concentrated at the bottom of the tube. When using it, please carefully blow it several times with a pipette.
7. In order to ensure the accuracy of the results, the standard curve should be made every time. Do not reuse diluted Standards, Concentrated Biotin-conjugated antibodies, and Concentrated HRP-conjugated Streptavidin.
8. The stop solution in this kit is an acidic solution. The operator should wear gloves and pay attention to the protection during use. Also avoid contact with skin and eyes during the operation. If inadvertently contact, please wash with plenty of water; for blood samples and other body fluid samples, please follow the relevant regulations of the National Biological Laboratory for safety protection.

Specimen requirements

1. Cell Culture Supernatant - Centrifuge cell culture media at 1000 X *g*, 20 min, 4°C. Remove impurities and cell debris, take the supernatant to detect.
2. Serum - Blood was solidifies naturally at room temperature. Centrifuge at 1000 X *g*, 20 min, 4°C, take the supernatant to detect.
3. Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge at 1000 X *g* for 15 min within 30 min after sample collection, and take the supernatant to detect. Avoid making use hemolysis, high blood lipid samples.
4. Tissue homogenates - It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolytic blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter. The homogenates are then centrifuged for 5-10 min at 5000 X *g* to get the supernatant.
5. Cell lysates: For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000 X *g*. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1 X 10^6 cells, add 150-250 µl of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10min at 1500 X *g* at 4°C. Remove the cell fragments, collect the supernatant to carry out the assay. Avoid repeated freeze-thaw cycles.

Notes:

1. Samples should be assayed within 7 days when stored at 4°C, otherwise samples must be divided up and stored at -20°C (≤1 month) or -80°C (≤3 months). Avoid repeated freeze-thaw cycles.

2. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

3. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

4. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.

5. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

Reagent preparation

1. Reagent was recovered to room temperature: The kit and the sample to be placed at room temperature 20 minutes before the experiment. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.
2. Prepare the washing solution: The volume of the diluted washing solution is pre-calculated, and then the concentrated washing solution (25X) is diluted to 1X working solution using the double-distilled water or deionized water.

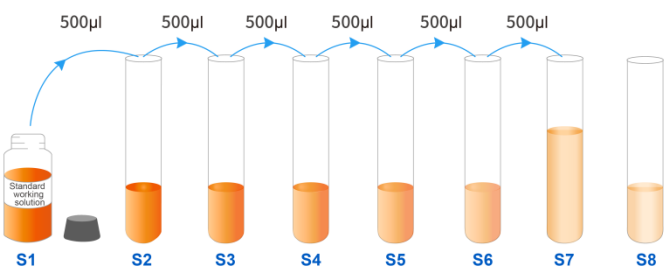
Note: If crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved.

3. Standard working solution: Centrifuge the standard at 10,000 X *g* for 1 min. Add 1.0 mL of Standard/Sample diluent, let it stand for 10 min and invert it gently several times. After it

dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 100 ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0 ng/mL. Dilution method: Take 7 EP tubes, add 500 µl of Standard/Sample diluent to each tube. Pipette 500 µl of the 100 ng/mL working solution to the first tube and mix up to produce a 50 ng/mL working solution. Pipette 500 µl of the solution from the former tube into the latter one according to these steps. The illustration below is for reference.

Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.

As shown below S1-S8.



4. Biotin-conjugated antibody working solution: The volume of the working solution is pre-calculated (50 µl/well), and then the concentrated Biotin-conjugated antibody (100X) is diluted to 1X working solution using the diluent SD2 (Mix thoroughly before dilution), please add to the reaction well within 30 min.
5. HRP-conjugated Streptavidin: The volume of the working solution is pre-calculated (100 µl/well), and then the concentrated HRP-conjugated Streptavidin (100X) is diluted to 1X working solution using the diluent SD3 (Centrifugation before dilution), please add it within 30

min.

6. Washing method:

Automatically wash the plate: Drain the liquid in the well of the microplate, pat dry on the thick absorbent paper, inject the washing solution 350 μl/well, inject and aspirate the interval for 30 seconds, and wash the plate 5 times.

Manually wash the plate: Drain the liquid in the well of the microplate, pat dry on the thick absorbent paper, add the washing solution 350 μl/well with a wash bottle, and sip the liquid in the well of the microplate after 1-2 min of rest, pat dry on the thick absorbent paper, and wash the plate 5 times.

Assay procedure

1. The number of the plates is pre-calculated, 30 min before experiment, take out the kit and restore it to room temperature.
2. Add 50 μl standards and samples in each reaction well, the standard needs to be duplicated, immediately add 50 μl biotin-conjugated antibody working solution to each reaction well, (If the sample concentration is higher than the detection range, sample should be dilution by the standard/sample diluent), incubated for 45 min at 37 °C.

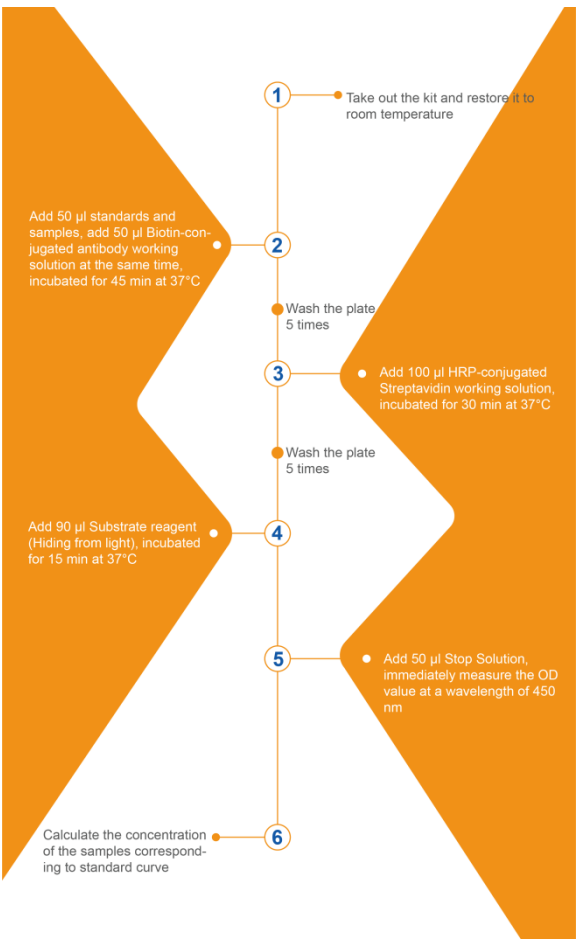
Note: Solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

3. Washing: Discard the liquid and dry it. Add 350 μl washing solution to each reaction well, and leave the washing solution after soak for 1~2 min. Repeat 4 times.
4. Add 100 μl HRP-conjugated streptavidin working solution to each reaction well, incubated for 30 min at 37 °C.
5. Washing: Add 300 μl washing solution to each reaction well, and leave the washing solution at

intervals of 30 seconds. Repeat 4 times.

6. Add 90 μl Substrate reagent (Hiding from light) to each reaction well, the color was developed at 37 °C for about 15 min.
7. Add 50 μl/well stop solution to each reaction well, immediately measure the OD value at a wavelength of 450 nm with a microplate reader (within 5 min).
8. Determine the OD value using the microplate reader at 450 nm.
9. With the standard concentration on the x-axis and the OD values on the y-axis, draw the standard curve with the four-parameter logistic model.
10. If the sample OD value is higher than the upper limit of the standard curve, it should be re-tested after appropriate dilution, and then multiplied by the dilution factor when calculating the concentration.

Step description



Sensitivity

The minimum detectable dose was 0.94 ng/mL.

Examination range

1.56-100 ng/mL

Specificity

This kit recognizes Universal FA/VB9(Folic Acid/Vitamin B9) in samples. No significant cross-reactivity or interference between Universal FA/VB9(Folic Acid/Vitamin B9) and analogues was observed.

Repeatability

Three different concentrations of samples were detected 20 times on the same board and 20 times on different boards. Within the plate or between the plates, the coefficient of variation is <10%.

Repeatability	**Intra-assay Precision**			**Inter-assay Precision**		
Sample	Low concentration	Middle concentration	High concentration	Low concentration	Middle concentration	High concentration
Test times	20	20	20	20	20	20
Meanng/mL	4.59	9.64	47.95	4.96	9.5	47.2
Standard	0.29	0.55	1.78	0.32	0.43	1.53

deviation						
C V (%)	6.32%	5.71%	3.71%	6.45%	4.53%	3.24%

Recovery

A known concentration of the target protein was added to 5 samples which were diluted to different multiples, and a recovery experiment was performed to obtain a recovery range and an average recovery rate.

Dilution factor	Serum (n=5)	EDTA plasma (n=5)	Cell culture media(n=5)	Recovery
Stock solution	93-107	88-102	91-107	Range (%)
	98	93	99	Average recovery (%)
1:2	91-107	91-103	93-110	Range (%)
	98	97	100	Average recovery (%)
1:4	93-106	91-102	95-108	Range (%)
	98	97	100	Average recovery (%)
1:8	91-106	90-102	95-111	Range (%)
	98	96	102	Average recovery (%)
1:16	88-101	88-101	93-106	Range (%)
	95	94	100	Average recovery (%)

Troubleshooting

Problem	Possible causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing
	Wells are not completely aspirated	Completely aspirate wells in between steps
Low signal	Insufficient incubation time	Ensure sufficient incubation time
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use
	Inadequate reagent volumes	Check pipettes and ensure correct preparation
	Improper dilution	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid coloring
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader
		Open the Microplate Reader ahead to preheat
Large CV	Inaccurate pipetting	Check pipettes
High background	Concentration of target protein is too high	Use recommended dilution factor

	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions
	Stop solution is not added	Stop solution should be added to each well before measurement