Elabscience®

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（本试剂盒仅供体外研究使用，不用于临床诊断!）

小鼠游离睾酮(F-TESTO)酶联免疫吸附测定试剂盒使用说明书

Mouse F-TESTO(Free Testosterone) ELISA Kit

产品货号：E-EL-M0518c

96T

使用前请仔细阅读说明书。如果有任何问题，请通过以下方式联系我们：

销售部电话 027-65022280，027-87854967

技术部电话 027-87526315

电子邮箱（销售）Perry@elabscience.cn

电子邮箱（技术）techsupport@elabscience.cn

QQ 客服 800110755

网址 www.elabscience.cn

具体保质期请见试剂盒外包装标签。

联系时请提供产品批号(见试剂盒标签)，以便我们更高效地为您服务

用途

该试剂盒用于体外定量检测血清、血浆或其他相关生物液体中天然F-TESTO浓度。

灵敏度、检测范围、特异性和重复性

●灵敏度：10pg/mL。

●检测范围：10–2000pg/mL。

●特异性：可检测天然 F-TESTO,且与孕酮、雌二醇、DHT 无明显交叉反应。

●重复性：板内，板间变异系数均<15%。

检测原理

本试剂盒采用竞争 ELISA 法。首先用羊抗兔包被微孔板，制成固相二抗，然后加入待测样本、辣根过氧化物酶标记的 F-TESTO 以及抗 F-TESTO 抗体，使之形成包被二抗-抗 F-TESTO 抗体-F-TESTO (HRP)复合物，标记 F-TESTO 的结合量与样品中的 F-TESTO 量成反比。加入显色底物(TMB)，TMB 在辣根过氧化物酶的催化下呈现蓝色，加终止液后变成黄色。用酶标仪在 450nm 波长处测 OD 值，F-TESTO 浓度与 OD450 值之间呈反比，通过绘制标准曲线计算出样品中 F-TESTO 的浓度。

试剂盒组成及保存

未拆封的试剂盒可在 4℃保存一周；如果一周以后才使用试剂盒，请拆开试剂盒按照下表中的条件分别保存各组分。

中文名称	规格	保存条件
ELISA 酶标板 Micro ELISA Plate	8 孔×12 条	4℃，可存放 6 个月
标准品一套(6 管) Reference Standard (6 tubes)	6 管 0.5mL	
检测抗体 Detection Ab	1 瓶 6mL	
浓缩洗涤液(20×) Concentrated Wash Buffer(20×)	1 瓶 15mL	
HRP 酶标记 F-TESTO 抗原 HRP-labeled F-TESTO	1 瓶 6mL	4℃(避光)，可存放 6 个月
底物溶液 A Substrate Reagent A	1 瓶 7mL	
底物溶液 B Substrate Reagent B	1 瓶 7mL	
反应终止液 Stop Solution	1 瓶 7mL	4℃
封板覆膜 Plate Sealer	5 张	
产品说明书 Manual	1 份	
质检报告 Certificate of Analysis	1 份	

说明：标准品一套的浓度分别为0 pg/mL, 10 pg/mL, 40 pg/mL, 160 pg/mL, 500 pg/mL, 2000 pg/mL。

所有试剂瓶盖须旋紧以防止蒸发和微生物的污染。

试剂体积以实际发货版说明书为准。相关试剂在分装时会比标签上标明的体积稍多一些，请在使用时量取而非直接倒出。

试验所需自备物品

1. 酶标仪(450nm波长滤光片)
2. 高精度移液器，EP管及一次性吸头：0.5-10μL, 2-20μL, 20-200μL, 200-1000μL
3. 37℃恒温箱，双蒸水或去离子水
4. 吸水纸

注意事项

1. 试验中请穿着实验服并戴乳胶手套做好防护工作。特别是检测血液或者其他体液样品时，请按国家生物试验室安全防护条例执行。
2. 刚开启的酶标板孔中可能会有少许水样物质，此为正常现象，不会对实验结果造成任何影响。暂时不用的板条应拆卸后放入备用铝箔袋，按照上述表格中保存条件存放。
3. 请勿重复使用已稀释过的试剂。酶标板及未用完的试剂原液按照上述表格中保存条件存放。
4. 检测使用的酶标仪需要安装能检测450±10nm波长的滤光片，光密度范围在0-3.5之间。
5. 不同批号的试剂盒组份不能混用。
6. 试验中所用的EP管和吸头均为一次性使用，严禁混用。

样品收集方法

(具体处理方法可参考官网：http://www.elabscience.cn)

1. 血清：全血样品于室温放置2小时或4℃过夜后于1000×g离心20分钟，取上清即可检测，收集血液的试管应为一次性的无内毒素试管。
2. 血浆：抗凝剂推荐使用EDTA-Na_2，样品采集后30分钟内于1000×g离心15分钟，取上清即可检测。避免使用溶血，高血脂样品。
3. 组织匀浆：用预冷的PBS (0.01M, 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1g的组织样品对应9mL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或反复冻融。最后将匀浆液5000×g离心5~10分钟，取上清检测。
4. 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×g离心5分钟后收集细胞；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10^6个细胞中加入150-200μL 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×g离心10分钟，取上清检测。
5. 细胞培养上清或其他生物体液：收集液体后1000×g离心20分钟，除去杂质及细胞碎片。取上清检测。

样品注意事项

1. 样品收集后若在1周内进行检测的可保存于4℃，若不能及时检测，请按一次使用量分装，冻存于-20℃(1个月内检测)，或-80℃(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检测抗体不匹配而出现不能检测的情况。

检测前准备工作:

1. 提前20分钟从冰箱中取出试剂盒，平衡至室温。读数前15分钟打开酶标仪预热。

2. 洗涤液: 将**浓缩洗涤液**用双蒸水稀释(1:19)。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℃水浴微加热使结晶完全溶解后再配制洗涤液。当日使用。

操作步骤(操作一览表见第 7 页)

1. 将预包被板从密封袋中取出，设一个空白对照孔，不加任何液体；每个标准点各设两孔，每孔加入相应**标准品** 50μL；其余每个检测孔直接加**待测样本** 50μL。
2. 加完后，立即每孔加入 **HRP 酶标记的 F-TESTO 抗原** 50μL (空白对照孔除外)，再按同样的顺序每孔加入**检测抗体** 50μL，充分混匀，贴上覆膜，置 37℃温育 1 小时。注意不要有气泡，加样时将样品加于酶标板底部，不要触及孔壁，轻轻晃动混匀。为保证实验结果有效性，每次实验请重新做标准曲线。
3. 甩尽孔内液体，每孔加**洗涤液** 350μL，浸泡 10 秒，吸去或甩掉酶标板内的液体，在厚的吸水纸上拍干。重复此洗板步骤 3 次。提示：此处与其他洗板步骤都可用洗板机。
4. 每孔加**底物溶液 A** 液 50μL，**底物溶液 B** 液 50μL，振荡混匀后，酶标板加上覆膜，37℃避光孵育 15 分钟左右。提示：根据实际显色情况酌情缩短或延长，但不可超过 30 分钟。当标准孔出现明显梯度时，即可终止。
5. 每孔加**终止液** 50μL, 终止反应。提示:终止液的加入顺序应尽量与底物溶液的加入顺序相同。
6. 立即用酶标仪在 450nm 波长测量各孔的光密度(OD 值)。

结果判断

1. 计算每组复孔的平均OD值。以浓度为横坐标，OD值为纵坐标，在双对数坐标纸上绘出四参数逻辑函数的标准曲线（作图时去掉空白组的值）。
2. 若样品OD值高于标准曲线上限，应适当稀释后重测。

问题分析

若实验效果不好，请及时对显色结果拍照，保存实验数据，保留所用板条及未使用试剂，然后联系我公司技术支持为您解决问题。同时您也可以参考以下资料：

问题描述	可能原因	相应对策
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
	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	
	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

操作概要

1. 在各孔中加入标准品或样品各 50μL,立即加入 50μL HRP 酶标抗原。然后各孔加入 50μL 检测抗体，37℃孵育 60 分钟
2. 洗涤 3 次
3. 加入 50μL 底物溶液 A 和底物溶液 B， 37℃孵育 15 分钟
4. 加入 50μL 终止液，立即在 450nm 波长处测量 OD 值
5. 结果计算

声明

1. 限于现有条件及科学技术水平，尚不能对所有原料进行全面的鉴定分析，本产品可能存在一定的质量技术风险。
2. 最终的实验结果与试剂的有效性、实验者的相关操作以及当时的实验环境密切相关，请务必准备充足的待测样品

Mouse F-TESTO(Free Testosterone) ELISA Kit

Catalog No: E-EL-M0518

96T

Intended use

This ELISA kit applies to the in vitro quantitative determination of MouseF-TESTO concentrations in serum, plasma and other biological fluids.

Specification

- Sensitivity: 10pg/mL.
- Detection Range: 10-2000pg/mL
- Specificity: This kit recognizes MouseF-TESTO in samples. No significant cross-reactivity or interference with progesterone, estradiol, dihydrotestosterone.
- Repeatability: Coefficient of variation is <15%.

Test principle

This ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with Goat Anti-Rabbit IgG, make solid-phase secondary antibody. And then add samples, horseradish peroxidase-labeled F-TESTO and anti-F-TESTO antibody, so as to form a coated secondary antibody - anti-F-TESTO antibody - HRP-labeled F-TESTO complex. The amount of bound labeled F-TESTO is inversely proportional to that of F-TESTO in the samples. The TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of MouseF-TESTO in the samples is then determined by comparing the OD of the samples to the standard curve

Kit components & Storage

An unopened kit can be stored at 4℃ for 1 week. If the kit is not used within 1 week, store the items separately according to the following conditions since the kit is received.

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	8 wells ×12 strips	4℃, 6 months
Reference Standard (6 tubes)	0.5 mL /tube	
Detection Ab	1 vial, 6 mL	
Concentrated Wash Buffer (20×)	1 vial, 15 mL	
HRP-labeled F-TESTO	1 vial, 6 mL	4℃(shading light), 6 months
Substrate Reagent A	1 vial, 7 mL	
Substrate Reagent B	1 vial, 7 mL	
Stop Solution	1 vial, 7 mL	4℃
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: A set of Standard concentrations is 0 pg/mL, 10 pg/mL, 40 pg/mL, 160 pg/mL, 500 pg/mL, 2000 pg/mL.

All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring.

Other supplies required

Microplate reader with 450 nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
37℃ Incubator
Deionized or distilled water
Absorbent paper
Loading slot for Wash Buffer

Note

1. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened ELISA Plate may appear to have a water-like substance, which is normal and will not have any impact on the experimental results.
3. Do not reuse the diluted working solution. The unspent solutions should be stored according to the storage conditions in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wave length at 450±10 nm.. The optical density should be within 0~3.5.
5. Do not mix or use components from other lots.
6. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.

Sample collection

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃before centrifugation for 20 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be non-endotoxin.

Plasma: Collect plasma using EDTA-Na_2 as anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8℃within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!

Tissue homogenates: It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed 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 or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5-10 min at 5000×g to get the supernatant.

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 centrifugal tube and centrifuge for 5min at 1000×g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 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×g at 4℃. Remove the cell fragments, collect the supernatant to carry out the assay. Avoid repeated freeze-thaw cycles.

Cell culture supernatant or other biological fluids: Centrifuge samples for 20 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay.

Note for sample:

1. Samples should be assayed within 7 days when stored at 4℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤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 detection antibody.

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. Preheat microplate reader for 15 min before OD measurement.
2. **Wash Buffer**: Dilute the concentrated wash solution to the working concentration using double distilled water (1:20), mix up. Put unused solution back at 4℃. Note: if crystals have formed in the concentrate, warm it in 40℃water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 1000×g for 1min, mix it thoroughly with a pipette.

Assay procedure (A brief assay procedure is on the 15th page)

1. Take out pre-coated plates. Set a Blank well, Do not add any liquid; Each Standard point set two wells, add 50μl of corresponding Standard per well; 50μl of Sample is added to the rest of each Sample well.
2. Immediately add 50 μl of HRP-labeled F-TESTO to each well (except Blank well). Then add 50 μl of Detection Ab to each well. The adding order of Detection Ab should be as the same as that of the HRP-labeled F-TESTO. Thorough mixing, cover with the Plate sealer provided in the kit. Incubate for 1hour at 37℃. Note: solutions are added to the bottom of micro ELISA plate well, avoid inside wall touching and foaming as possible.
3. Aspirate or decant the solution from each well， add 350μL of **wash buffer** to each well. Soak for 10 sec and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times in total. Note: a microplate washer can be used in this step and other wash steps
4. Add 50μL of Substrate A and Substrate B to each well, mix fully. Incubate for about 15 min at 37℃. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.
5. Add 50μLof **Stop Solution** to each well. Note: the order to add stop solution should be the same as the substrate solution.
6. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Troubleshooting

Problem	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 onthe Microplate reader.
		Open the Microplate Reader ahead to pre-heat.
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.

Summary

1. Add 50μL of standard or sample to each well. Immediately add 50μL of HRP-labeled F-TESTO to each well. Then add 50μL of Detection Ab to each well. Incubate for 1 hour at 37℃
2. Aspirate and wash 3 times
3. Add 50μL of Substrate A and Substrate B to each well. Incubate 15 min at 37℃
4. Add 50μL Stop Solution. Read at 450nm immediately
5. Calculation of results.

Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. The final experimental results will be closely related to the validity of products, operational skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions!
4. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
5. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
6. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipments, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.