



(For scientific research use only, not for clinical diagnosis!)

Rat elongation factor very long chain fatty acid-like protein 2

(ELOVL2) ELISA kit instruction manual Product number:

BY-ER338994 Specifications: 48T/96T Detection range: 0.312

ng/mL- 10 ng/mL.

Sensitivity: The lowest detectable dose is less than 0.1 ng/mL.

Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%.

Recovery rate: The recovery rate is between 85%-115%.

Specificity: This kit recognizes native and recombinant rat elongation factor extremely long-chain fatty acid-like protein 2 (ELOVL2) and has no crossover with structural analogs.

Stability: Stored at 2°C-8°C, validity period is 6 months.

Purpose: Used to detect the concentration of rat elongation factor very long chain fatty acid-like protein 2 (ELOVL2) in samples such as serum, plasma, cell culture supernatant and tissue.

Please read the instructions carefully before use. If you have any questions, please contact us through the following methods: Official hotline: 025-5229-

8998 Sales department phone: 13914481711 Technical phone: 15950492658

Company website: www.byabscience.cn For the specific shelf life, please refer to the outer packaging label of the kit. Please use the kit within the shelf life.

Nanjing BYabscience technology Co.,Ltd

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Official hotline: 025-5229-8998

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When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.



Experimental principle

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). Add rat elongation factor very long chain fatty acid-like protein 2 (ELOVL2) calibrator to the microwell microplate pre-coated with anti-rat elongation factor very long chain fatty acid-like protein 2 (ELOVL2) antibody (solid-phase antibody) and the sample to be tested, then add HRP-labeled anti-rat elongation factor extremely long chain fatty acid-like protein 2 (ELOVL2) antibody (enzyme-labeled antibody), after incubation and sufficient washing, remove unbound components, and place in a microplate. A sandwich complex of solid-phase antibody-antigen-enzyme-labeled antibody is formed on the solid surface. Add substrates A and B. The substrates are catalyzed by HRP to produce a blue product, which is finally converted to yellow under the action of the stop solution (acidic solution). The absorbance (OD value) was measured at a wavelength of 450 nm using a microplate reader. The absorbance (OD value) was positively correlated with the concentration of rat elongation factor extremely long chain fatty acid-like protein 2 (ELOVL2) in the sample to be tested. The concentration of the rat elongation factor extremely long chain fatty acid-like protein 2 (ELOVL2) in the sample can be calculated by fitting the calibrator curve.

Experimental schematic diagram



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Kit components and storage: Store unopened kits at 2-8 degrees Celsius. Do not use expired kits.

Components	48-well configuration	96-well configuration	Store after opening
Pre-coated enzyme	48T	96T	2-8°C 14 days
Standard product	0.3mL*6 tubes	0.3mL*6 tubes	2-8°C 14 days
sample diluent	3ml	6ml	2-8°C 180 days
HRP labeled antibodies	5ml	10ml	2-8°C 14 days
Chromogenic substrate	3ml	6ml	2-8°C 180 days
Chromogenic substrate	3ml	6ml	2-8°C 180 days
stop solution	3ml	6ml	2-8°C 180 days
20×Lotion	15ml	25ml	2-8°C 180 days
sealing film	2 sheets	2 sheets	
manual	1 serving	1 serving	
Ziplock bag	1	1	

The concentrations of calibrators are: 10, 5, 2.5, 1.25, 0.625, 0.312 ng/mL.

Note: 1: Before use, please check whether the label and quantity of

the reagents in the kit are consistent with the table.

2: If the components of the kit need to be used again, please ensure that they have

not been contaminated since the last use. 3: If the enzyme plate is not used up in a

single time, remember to seal it and store it at 2-8°C.

Prepare your own test equipment required for the test (not provided, but can assist in

1) 能够检测 450 nm 吸光度的酶标仪 2)

移液器及枪头、加样槽 3) 37°C恒温箱或

水浴锅 4) 准备试剂用的试管、离心管、

量筒等 5) 蒸馏水或去离子水

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监督电话: 15950492658



6) 涡旋振荡器、微孔板振荡器

注意事项 1) 仅供科研使用，不得用于临床诊断。

- 2) 在试剂盒标示的有效期内使用，过期产品不得使用。
- 3) 跟其他厂家的试剂盒或者组分不能混用，使用试剂盒配套的样品稀释液。
- 4) 如果样本值高于最高标准品浓度值，请将样本适当稀释后，再重新测定。
- 5) 待测样本中存在的人抗鼠等异嗜抗体会干扰检测结果，检测前，请排出该因素。
- 6) 通过其他方法得到的检测结果，与本试剂盒测定结果不具有直接的可比性。
- 7) 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。
- 8) 严格按照规定的时间和温度进行温育以保证准确结果。所有试剂都必须在使用前达到室温 20-25°C。使用后立即冷藏保存试剂。
- 9) 洗板不正确可以导致不准确的结果。在加入底物前确保尽量吸干孔内液体。温育过程中不要让微孔干燥掉。
- 10) 消除板底残留的液体和手指印，否则影响 OD 值。
- 11) 底物显色液应呈无色或很浅的颜色。
- 12) 避免试剂和标本的交叉污染以免造成错误结果。
- 13) 在储存和温育时避免强光直接照射。
- 14) 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。建议使用时提前 15 分钟预热。
- 15) 试验中所用的 EP 管和吸头均为一次性使用，严禁混用。



样品的准备和保存

以下只是列出样品采集和保存的一般指南。所有样本采集保存过程中，不得使用叠氮钠做为防腐剂。样品如果不立即分析，应分装后冷冻保存，且避免反复冻融。

细胞培养上清——离心去除沉淀，立即分析或分装后-20℃冷冻保存。

血清——用干净试管收集血液，室温凝固 30 分钟，离心 2000×g 20 分钟，收集血清。立即分析或分装后-20℃冷冻保存。

血浆——采用肝素、柠檬酸盐或 EDTA 抗凝，抽血后 30 分钟内在 2-8℃离心 2000×g 20 分钟。为消除血小板的影响，建议在 2-8℃进一步离心 10000×g 10 分钟。立即分析或分装后-20℃冷冻保存。

细胞裂解液——对于贴壁细胞，去除培养液，用 PBS、生理盐水或无血清培养液洗一遍。加入适量裂解液，用枪吹打数下，使裂解液和细胞充分接触。通常 10 秒后，细胞就会被裂解。对于悬浮细胞，离心收集细胞，用 PBS、生理盐水或无血清培养液洗一遍。加入适量裂解液，用枪吹打把细胞吹散，用手指轻弹以充分裂解细胞。充分裂解后，10000—14000×g 离心 3-5 分钟，取上清。立即分析或分装后-20℃冷冻保存。

组织匀浆——用预冷的 PBS (0.01M, pH=7.4)冲洗组织，去除残留血液（匀浆中裂解的红细胞会影响测量结果），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，于冰上充分研磨。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。最后将匀浆液于 5000×g 离心 5~10 分钟，取上清检测。

尿液——用无菌管收集，离心 2000×g 20 分钟。仔细收集上清。如有沉淀形成，应再次离心。



试剂准备 1、使用前，所有的组分都要至少复温 60min，确保充分复温到室温。

2、浓缩洗涤液：从冰箱取出的浓缩洗涤液，会有结晶产生，这属于正常现象，水浴加热使结晶完全溶解。浓缩洗涤液与蒸馏水，按 1:20 稀释，即 1 份的浓缩洗涤液，添加 19 份的蒸馏水。3、底物：底物液 A 和 B，在使用前，按 1:1 体积充分混合，混合后 15 分钟内使用。

操作程序 所有试剂和组分都先恢复到室温，标准品、质控品和样品，建议做复孔。

1. Prepare the working solution of various components of the kit according to the method described in the previous instructions.
2. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.
3. Set up standard wells, 0 value wells, blank wells and sample wells. Add 50 μL of standards of different concentrations to each of the standard wells. Add 50 μL of sample diluent to the 0 value well. Do not add any to the blank well. Add 50 μL of the sample to be tested to the sample well. .
4. In addition to the blank wells, add 100 μL of horseradish peroxidase (HRP)-labeled detection antibody to the standard wells, 0 value wells and sample wells.
5. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 60 minutes.
6. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing liquid, let it stand for 20 seconds, shake off the washing liquid, pat dry on absorbent paper, repeat this 5 times. If you use an automatic plate washer, please wash the plate according to the operating procedures of the plate washer. Adding a soaking program for 30 seconds can improve the detection accuracy. After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper. (Tip: In order to obtain ideal experimental results, the residual liquid must be completely removed. After

washing the plate, please proceed to the next step immediately and do not let the microplate dry.) 7. Mix substrates A and B at a volume of 1:1 Mix thoroughly and add 100 μ L of substrate mixture to all wells. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 15 minutes.

8. Add 50 μ L of stop solution to all wells, and read the absorbance (OD value) of each well on a 450nm wavelength microplate reader.

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[Operation flow chart]



1. 对应板孔中加入50 μ L标准品工作液或样本后，立即每孔加入100ulHRP酶标抗体工作液，37 $^{\circ}$ C孵育60分钟



2. 弃掉板内液体，洗板5次



3. 每孔加入底物A溶液50ul，底物B溶液50ul



4. 每孔加入50 μ L终止液



5. 立即在450nm波长下读数，处理数据



Result calculation

9. Use the concentration of the standard substance as the abscissa and the corresponding absorbance (OD value) as the ordinate. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. [Calculate using ELISA Calc software. It is recommended to use four-parameter fitting for the standard curve, but it is not the only fitting method]
10. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to determine the final value of the sample. concentration. Note: Experimenters need to establish a standard curve based on their own experiments. For each test, a standard curve must be established for each enzyme plate. The following curves are for reference only!



(Schematic diagram of the music, for reference only)

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[Problem Analysis] If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the used strips and unused reagents, and then contact our company's technical support to solve the problem for you. At the same time, you can also refer to the following information:

[Questions and Answers]

Problem description	Possible reasons	Corresponding countermeasures Corresponding countermeasures
standard curve gradient difference	Incorrect liquid aspiration or	Check pipettes and tips
	Equilibration time is too short	Ensure sufficient balancing time
	Incomplete washing	Ensure the washing time and number of washings and the amount of liquid added to each hole
Very weak or colorless	Incubation time too short	Ensure adequate incubation time
	The experimental temperature is incorrect	Use recommended experimental temperatures
	Insufficient reagent volume or missing addition	Check the liquid aspirating and adding process to ensure that all reagents are added in order and in
	Incorrect dilution	
Enzyme label inactivation or substrate failure	Mix enzyme conjugate and substrate and check by rapid color development	
Reading value is low	Microplate reader settings are incorrect	Check the wavelength and filter
		Turn on the microplate reader and preheat it in advance
Large coefficient of variation	Adding fluid incorrectly	Check the filling situation
High background value	The working concentration of the	Use the recommended dilution
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked;
	The lotion is contaminated	Prepare fresh lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to
	Not terminated before reading	Stop solution should be added to

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statement

1. Due to the current conditions and scientific and technological level, it is not possible to conduct comprehensive identification and analysis of all raw materials. This product may have certain quality and technical risks.
2. This kit removes/reduces some endogenous interfering factors in biological samples during the development process. Not all possible influencing factors have been removed.
3. The final experimental results are closely related to factors such as the effectiveness of the reagents, the relevant operations of the experimenter, and the experimental environment at the time. Our company is only responsible for the kit itself and is not responsible for the sample consumption caused by the use of the kit. Please use The user should fully consider the possible usage of the sample and reserve sufficient samples before use.
4. In order to achieve good experimental results, please only use the reagents provided in our company's kits, do not mix products from other manufacturers, and operate in strict accordance with the instructions.
5. Due to incorrect reagent preparation and microplate reader parameter settings during the operation, abnormal results may occur. Please read the instructions carefully and adjust the instrument before the experiment.
6. Even if operated by the same personnel, different results may be obtained in two independent experiments. In order to ensure the reproducibility of the results, it is necessary to control every step of the experimental process.

7. The kits will undergo strict quality inspection before shipment. However, due to factors such as transportation conditions, differences in experimental equipment, etc., user test results may be inconsistent with factory data.

8. This kit has not been compared with similar kits from other manufacturers or products using different methods to detect the same target, so inconsistent test results cannot be ruled out.

9. The kit is for research use only. If it is used for clinical diagnosis or any other purpose, our company will not be responsible for any problems arising therefrom, nor will we assume any legal liability.

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