



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

Mouse epidemic diarrhea virus IgE antibody (PEDV-IgE)

ELISA kit instruction manual Product number: BY-

EM224244 Specifications: 48T/96T

**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
Contact email: 3224949330@qq.com Company website:
www.byabscience.cn For specific shelf life, please see the reagents Box
packaging label. Please use the kit within the shelf life.**

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

Nanjing BYabscience technology Co.,Ltd

Website: www.byabscience.cn

Official hotline: 025-5229-8998

Supervision phone number:



Kit performance Physical properties: Each liquid component is clear and transparent, with no precipitation or floc. Microplate aluminum foil bags should be vacuum packed without damage or leakage.

Negative control OD value: less than 0.2.

Positive control OD value: greater than 0.8.

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 mouse epidemic diarrhea virus IgE antibodies (PEDV-IgE) and has no crossover with structural analogs.

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

Purpose: Used for the qualitative detection of mouse epidemic diarrhea virus IgE antibodies (PEDV-IgE) in samples such as serum, plasma, cell culture supernatants, and tissues.

Shelf life: Stored at 2°C-8°C, valid for 6 months.

Experimental principle

The kit uses an indirect enzyme-linked immunosorbent assay (ELISA). To the microwells pre-coated with mouse epidemic diarrhea virus IgE antibody (PEDV-IgE) capture antigen, add the specimen, negative and positive controls in sequence, then add the HRP-labeled detection antibody, incubate and wash thoroughly. The substrate TMB is used for color development. TMB is converted into blue under the catalysis of peroxidase, and converted into the final yellow under the action of acid. The depth of the color is positively correlated with the amount of mouse epidemic diarrhea virus IgE antibodies (PEDV-

IgE) in the sample. Use a microplate reader to measure the absorbance (OD value) at a wavelength of 450 nm to determine negative and positive.

Nanjing BYabscience technology Co.,Ltd

Website: www.byabscience.cn

官方热线: 025-5229-8998

监督电话: 15950492658



试剂盒组分与保存 未开封的试剂盒保存在 2-8 度，不得使用过期试剂盒。

组分	48 孔配置	96 孔配置	开封后储存
预包被酶标板	48T	96T	2-8℃14 天
阴性对照	0.3mL	0.3mL	2-8℃14 天
阳性对照	0.3mL	0.3mL	2-8℃14 天
样本稀释液	3 ml	6 ml	2-8℃180 天
HRP 标记抗体	5 ml	10 ml	2-8℃14 天
显色底物 A	3 ml	6 ml	2-8℃180 天
显色底物 B	3 ml	6 ml	2-8℃180 天
终止液	3 ml	6 ml	2-8℃180 天
20×洗液	15 ml	25 ml	2-8℃180 天
封板膜	2 张	2 张	
说明书	1 份	1 份	
自封袋	1 个	1 个	

注意：1：使用前请检查试剂盒中试剂的标签和数量与表格是否一致。

2：如果试剂盒的组份需要再次使用，请确保上一次使用之后没有被污染。

3：酶标板单次未使用完，要谨记密封放到 2-8℃保存。

试验所需自备试验器材 (不提供，但可协助购买)

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℃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 6) 涡旋振荡器、微孔板振荡器

Nanjing BYabscience technology Co.,Ltd

网址: www.byabscience.cn

官方热线: 025-5229-8998

监督电话: 15950492658



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，请按国家生物实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℃。使用后立即冷藏保存试剂。
- 9) Improper plate washing can lead to inaccurate results. Make sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.
- 10) Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.
- 11) The substrate chromogenic solution should be colorless or very light in color.
- 12) Avoid cross-contamination of reagents and specimens to avoid erroneous results.
- 13) Avoid direct exposure to strong light during storage and incubation.
- 14) The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of $450\pm 10\text{nm}$, and the optical density range is between 0-3.5. It is recommended to preheat 15 minutes in advance before use.
- 15) The EP tubes and suction tips used in the test are single-use and are strictly prohibited from mixing.

Nanjing BYabscience technology Co.,Ltd

Website: www.byabscience.cn

Official hotline: 025-5229-8998

Supervision phone number:



Sample preparation and storage

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide must not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

Cell culture supernatant - centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.

Serum - Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.

Plasma—anticoagulate with heparin, citrate, or EDTA, and centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood draw. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.

Cell lysis buffer - For adherent cells, remove the culture medium and wash with PBS, normal saline or serum-free culture medium. Add an appropriate amount of lysis solution and pipet several times with a gun to fully contact the lysate and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them once with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, blow the cells with a gun, and flick them with your fingers to fully lyse the cells. After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.

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。

Nanjing BYabscience technology Co.,Ltd

网址: www.byabscience.cn

官方热线: 025-5229-8998

监督电话: 15950492658



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。

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、按前面说明书描述的方法，配制好试剂盒各种组分的工作液。
- 2、从铝箔袋中取出所需板条，剩余的板条用自封袋密封放回冰箱。
- 3、设置标准品孔和样本孔，标准品孔各加不同浓度的标准品 50 μ L；
- 4、样本孔中加入待测样本 50 μ L；空白孔不加。
- 5、除空白孔外，标准品孔和样本孔中每孔加入辣根过氧化物酶（HRP）标记的检测抗体 100 μ L，用封板膜封住反应孔，37 $^{\circ}$ C 水浴锅或恒温箱温育 60min。
- 6、弃去液体，吸水纸上拍干，每孔加满洗涤液（350 μ L），静置 1min，甩去洗涤液，吸水纸上拍干，如此重复洗板 5 次（也可用洗板机洗板）。
- 7、每孔加入底物 A、B 各 50 μ L，37 $^{\circ}$ C 避光孵育 15min。
- 8、每孔加入终止液 50 μ L，15min 内，在 450nm 波长处测定各孔的 OD 值。

[检测结果的解释]

- 1、阴性对照 OD 值：小于 0.2。
- 2、阳性对照 OD 值：大于 0.8。

3. Positive judgment (Cut-Off value): If the negative control OD value is +0.25, and the sample OD value is greater than the threshold, it is judged as positive, otherwise, it is negative.

Nanjing BYabsience technology Co.,Ltd

Website: www.byabsience.cn

Official hotline: 025-5229-8998

Supervision phone number:



[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	
Reading value is low	Microplate reader settings are incorrect	Mix enzyme conjugate and substrate and check by rapid color development
		Check the wavelength and filter
Large coefficient of variation	Adding fluid incorrectly	Turn on the microplate reader and preheat it in advance
		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

Nanjing BYabscience technology Co.,Ltd

Website: www.byabscience.cn

Official hotline: 025-5229-8998

Supervision phone number:



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. 为了达到好的实验结果，请只使用本公司试剂盒内提供的试剂，不要混用其他制造商的产品，严格按照说明书操作。

5. 由于操作过程中试剂制备以及酶标仪参数设置不正确，可能导致结果异常，实验前请仔细阅读说明书并调整好仪器。

6. 即使是相同人员操作也可能在两次独立实验中得到不同的结果，为保证结果的重现性，需要控制实验过程中每一步的操作。

7. 试剂盒发货前会经过严格的质检，然而，因为运输条件、实验设备差异等等因素影响，用户检测结果可能跟出厂数据不一致。

8. 本试剂盒未与其他厂家同类试剂盒或不同方法检测同一目的物的产品进行对比，所以不排除检测结果不一致的情况。

9. 试剂盒仅供研究使用，如将其用于临床诊断或任何其他用途，我公司将不对因此产生的问题负责，亦不承担任何法律责任。

Nanjing BYabscience technology Co.,Ltd

网址：www.byabscience.cn

官方热线：025-5229-8998

监督电话：15950492658