(仅供科研使用，不得用于临床诊断!)

猴环磷酸腺苷(cAMP)ELISA 试剂盒

使用说明书 规格：48T/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：

官方热线：025-5229-8998 销售部电话：13914481711 技术电话：

15950492658 联系邮箱：3224949330@qq.com 公司网址：

www.byabscience.cn 具体保质期请见试剂盒外包装标签。请在保质期内使

用试剂盒。

联系时请提供产品货号、生产日期（见盒签），以便我们更高效为您服务。

试剂盒性能 检测范围：1.25 ng/mL– 20 ng/mL。

灵敏度：最低检出剂量小于 0.1 ng/mL。

精密度：批内变异系数 CV%小于 10%；批间变异系数 CV%小于 15%。

回收率：回收率在 85%-115%之间。

特异性：本试剂盒识别天然和重组猴环磷酸腺苷(cAMP)，与结构类似物无交叉。

稳定性：2℃-8℃保存，有效期 6 个月。

用途：用于检测血清、血浆、细胞培养上清液和组织等样本中猴环磷酸腺苷(cAMP)的浓度。

保质期：2℃-8℃保存，有效期 6 个月。

实验原理

试剂盒采用酶联免疫分析方法。采用生物素标记 cAMP，纯化的抗 cAMP 抗体包被微孔板，在竞争抑制反应中，一定量的固相抗体与生物素标记 cAMP 及非标记抗原（校准品或标本）进 行抑制竞争反应，抗体与生物素标记的 cAMP 结合量受非标记抗原量所抑制，非标记抗原量多, 抗体与生物素标记的 cAMP 结合就少，反之结合就多；反应平衡后，形成固相抗体-生物素化 cAMP，再加入酶标记的亲和素，形成固相抗体-生物素化 cAMP-酶标-亲合素复合物。经加底 物显色后，用酶标仪在 450nm 波长下测定吸光度（OD 值）。随着 cAMP 浓度的升高，OD 值 逐渐下降呈良好的线性关系。本试剂盒具有灵敏度高、特异性强、重复性好、操作简单、快速 等特点，对血清中 cAMP 的减少或升高有可靠的检出性能。

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**
标准品	0.3mL*6 管	0.3mL*6 管	**2-8℃14 天**
样本稀释液	3 ml	6 ml	2-8℃180 天
生物素化抗原	3 ml	6 ml	**2-8℃14 天**
HRP 标记亲和素	3 ml	6 ml	2-8℃180 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	

校准品浓度依次为：20 、10 、5 、2.5 、1.25 、0 ng/mL。

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

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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) Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

8) Carry out incubation strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.

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±10nm, 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 tips used in the test are single-use and are strictly prohibited from mixing.

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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, physiological 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 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。

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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、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；每个校准品设 2 孔，每孔加入对应校准品 50μl；其余每个检测孔直接加待测血清或质控品 50μl。

4、除空白孔外所有孔加入生物素化抗原 50μl，混匀，贴上封板膜，置 37℃温育 60 分钟。

5、手工洗板：弃去孔内液体，洗涤液注满各孔，静置 10 秒甩干，重复 3 次后拍干。洗板机洗板：选择洗涤 3 次程序洗板后拍干。

（提示：为获得理想的实验结果，必须彻底移除残留液体。洗板完成之后，请立即进行下步操作，不要让微孔板干燥。）6、每孔加入酶标亲和素 50μl（空白对照孔除外），混匀，贴上封板膜，置 37℃温育 30 分钟。

7、手工洗板：弃去孔内液体，洗涤液注满各孔，静置 10 秒甩干，重复 3 次后拍干。洗板机洗板：选择洗涤 3 次程序洗板后拍干。

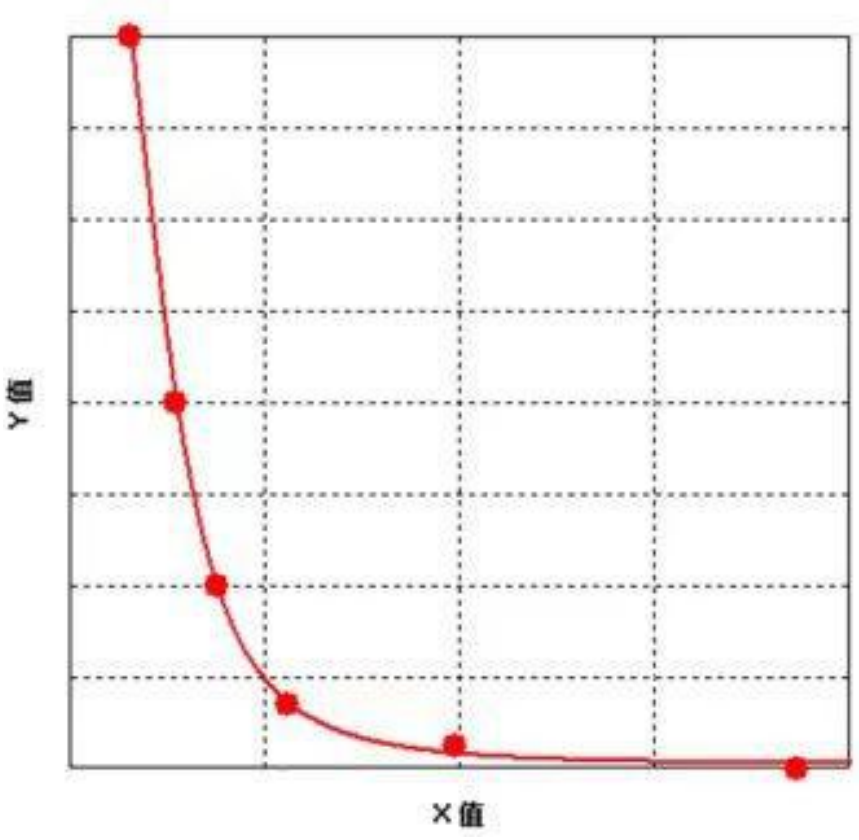
8. Add 50 μl of chromogen A and 50 μl of chromogen B to each well. After shaking and mixing, place at 37°C to develop color in the dark for 15 minutes. Add 50 μl of stop solution to each well.

9. Use a microplate reader to read, take the wavelength of 450nm, first use the blank control well to adjust the zero point, and then measure the optical density value (OD value) of each well.

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. [Calculation using ELISA Calc software]

10. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample. 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)

[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
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	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