



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

Human Myristylated Alanine Rich Protein Kinase C Substrate

(MARCKS) ELISA Kit Instructions for Use Product Catalog

Number: BY-EH1102057 Specifications: 48T/96T Detection Range:

0.25 ng/mL– 8 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 human myristylated alanine-rich protein kinase C substrates (MARCKS) without crossover to structural analogs.

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

Purpose: Used to detect the concentration of human myristylated alanine-rich protein kinase C substrate (MARCKS) 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.**

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Official hotline: 025-5229-8998

Supervision phone number:



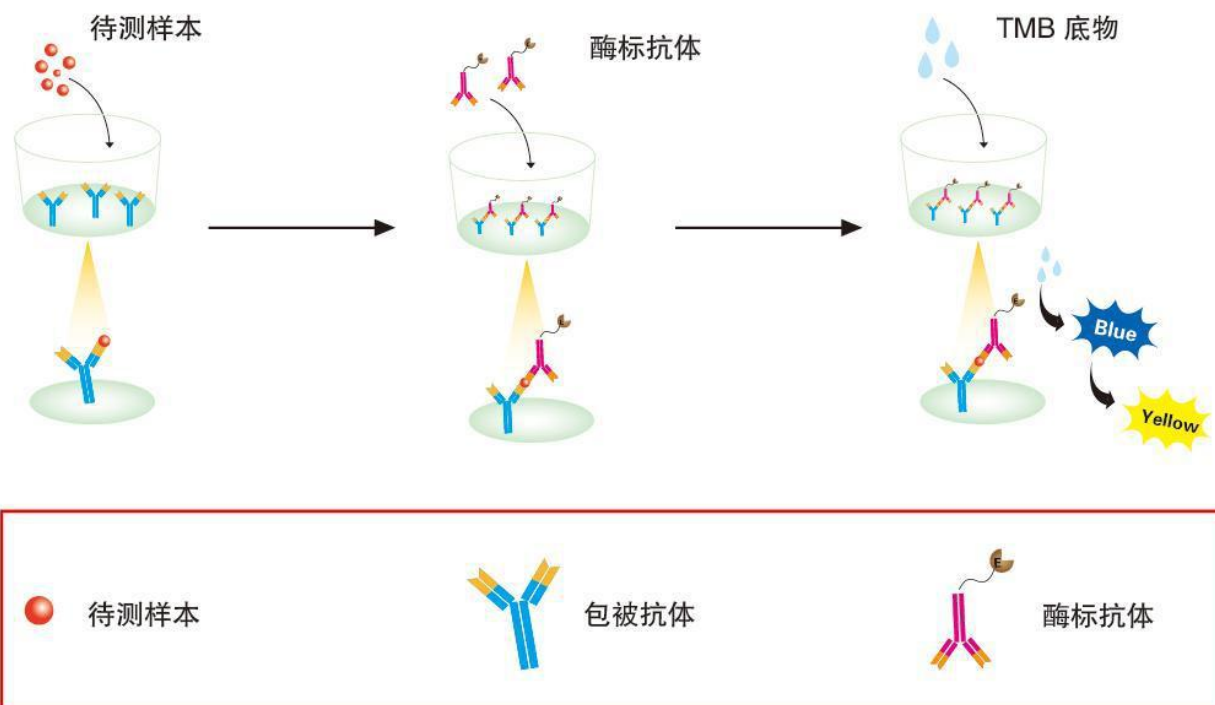
When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.



Experimental principle

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In the microwell microtiter plate pre-coated with anti-human myristylated alanine-rich protein kinase C substrate (MARCKS) antibody (solid-phase antibody), add human myristylated alanine-rich protein kinase C substrate (MARCKS) calibrator and sample to be tested, then add HRP-labeled anti-human myristylated alanine-rich protein kinase C substrate (MARCKS) antibody (enzyme-labeled antibody), after incubation and thorough washing, remove unbound components, and form a sandwich complex of solid-phase antibody-antigen-enzyme-labeled antibody on the solid surface of the microplate. Adding substrates A and B, the substrates are catalyzed by HRP to produce a blue product, which is finally converted into yellow under the action of the stop solution (acidic solution). The absorbance (OD value) was measured on a microplate reader at a wavelength of 450 nm, and the absorbance (OD value) was positively correlated with the concentration of human myristylated alanine-rich protein kinase C substrate (MARCKS) in the sample to be tested. The concentration of human myristylated alanine-rich protein kinase C substrate (MARCKS) 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 plate	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 A	3ml	6ml	2-8°C 180 days
Chromogenic substrate B	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: 8, 4, 2, 1, 0.5, 0.25 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) Microplate reader capable of detecting absorbance at 450 nm 2) Pipette, pipette tip, and sample addition tank 3) 37°C incubator or water bath 4) Test tubes, centrifuge tubes, measuring cylinders, etc. for preparing

reagents 5) Distilled water or deionized

water Ionized water

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6) Vortex shaker, microplate shaker

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- 2) Use within the validity period marked on the kit. Expired products must not be used.
- 3) Do not mix with kits or components from other manufacturers. Use the sample diluent provided with the kit.
- 4) If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then re-measure.
- 5) Human anti-mouse and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.
- 6) The test results obtained by other methods are not directly comparable to the test results of this kit.
- 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 $450\pm 10\text{nm}$, 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 tips used in the test are single-use and are strictly prohibited from mixing.

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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 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.

Tissue homogenate - rinse the tissue with pre-cooled PBS (0.01M, pH=7.4) to remove residual blood (lysed red blood cells in the homogenate will affect the measurement results), weigh and cut the tissue into pieces. Mix the minced tissue with the corresponding volume of PBS (generally

at a weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS. The specific volume can be adjusted appropriately according to experimental needs and recorded. It is recommended to add Protease inhibitor) was added to a glass homogenizer and ground thoroughly on ice. In order to further lyse tissue cells, the homogenate can be sonicated or repeatedly frozen and thawed. Finally, centrifuge the homogenate at $5000 \times g$ for 5 to 10 minutes, and take the supernatant for detection.

Urine - Collect in sterile tubes and centrifuge at $2000 \times g$ for 20 minutes. Carefully collect the supernatant. If a precipitate forms, centrifuge again.

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**Reagent preparation 1. Before use, all components must be rewarmed**

for at least 60 minutes to ensure sufficient rewarming to room temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the crystals. Concentrated detergent and distilled water, dilute 1:20, that is, 1 part of concentrated detergent, add 19 parts of distilled water. 3. Substrate: Substrate solutions A and B, mix thoroughly at a volume of 1:1 before use, and use within 15 minutes after mixing.

Operating procedures: Return all reagents and components to room temperature first. It is recommended to do duplicate holes for standards, quality control materials and samples.

- 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、0 值孔、空白孔和样本孔，标准品孔各加不同浓度的标准品 50 μ L，0 值孔加样本稀释液 50 μ L，空白孔不加，样本孔加待测样本 50 μ L。
- 4、除空白孔外，标准品孔、0 值孔和样本孔，加入辣根过氧化物酶（HRP）标记的检测抗体 100 μ L。
- 5、用封板膜盖住反应板，37 $^{\circ}$ C 水浴锅或恒温箱避光孵育 60min。
- 6、揭开封板膜，弃去液体，吸水纸上拍干，每孔加满洗涤液，静置 20S，甩去洗涤液，吸水纸上拍干，如此重复 5 次。若使用自动洗板机，请按洗板机操作程序进行洗板，添加浸泡 30s 的程序，可以提高检测的精度。洗板结束，加底物前，要在干净不掉屑的纸上，充分拍干反应板。（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。）7、将底物 A 和 B 按 1:1 体积充分混合，所有孔中加入底物混合液 100 μ L。用封板膜盖住反应板，37 $^{\circ}$ C 水浴锅或恒温箱避光孵育 15min。

8、所有孔加入终止液 50 μ L，在 450nm 波长酶标仪上读取各孔吸光度（OD 值）。

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官方热线：025-5229-8998

监督电话：15950492658



操作流程



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100 μ L HRP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50 μ L，底物B溶液50 μ L



4. 每孔加入50 μ L终止液

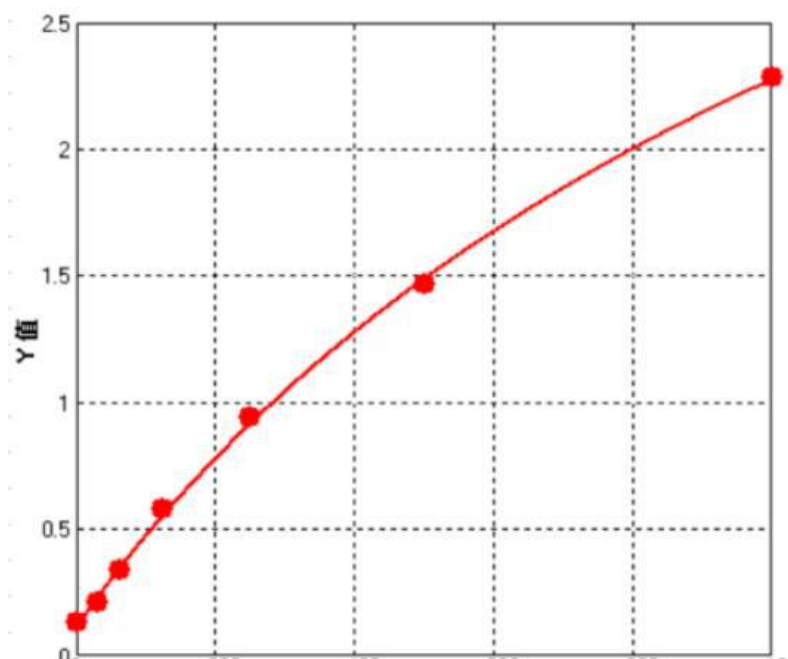


5. 立即在450nm波长下读数，处理数据



结果计算

9、以标准品浓度做为横坐标，对应的吸光度（OD 值）作为纵坐标，利用计算机软件，采用四参数 Logistic 曲线拟合（4-p1），创建标准曲线方程，通过样本的吸光度（OD 值），利用方程计算样品的浓度值。【用 ELISA Calc 软件计算，标曲建议使用四参数拟合，但不是唯一拟合方式】10、如果样品被稀释，通过上述方法测得的浓度值，要乘以稀释倍数，才是样品的最终浓度。注意：实验者需根据自己的实验建立标准曲线。每次检测，每块酶标板都必须设立标准曲线。以下曲线仅供参考！



（标曲示意图，仅供参考）

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[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：[问题解答]

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
	平衡时间太短	保证充足的平衡时间
	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



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