优品生物
UpingBio

(仅供科研使用，不得用于临床诊断!)

马 D-二聚体(D2D)定量检测试剂盒（ELISA）

使用说明书 规格：48T/96T 货号：YPJ1487

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：400-999-8863 技术电话：18358180525 邮箱：

UpingBio@163.com 公司网址：www.upingbio.com 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 物理性能：各液体组分澄清透明、无沉淀或者絮状物。微孔板铝箔袋应真空包装，无破损漏气。

标准曲线线性：校准品剂量反应曲线相关系数 r 值，大于等于 0.9900。

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

灵敏度：最低检出剂量小于 39.062 ng/ml。

回收率：回收率在 85%-115%之间。

敏感性：本试剂盒识别天然和重组马 D-二聚体(D2D)，与结构类似物无交叉。

稳定性：2℃-8℃保存，有效期 6 个月。

检测范围：312.5 ng/ml-5000 ng/ml。

用途：用于检测血清、血浆、细胞培养上清液等样本中马 D-二聚体(D2D)的浓度。

实验原理 试剂盒采用酶联免疫分析方法。采用生物素标记马 D-二聚体(D2D)，纯化的抗马 D-二聚体(D2D) 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马 D-二聚体(D2D)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马 D-二聚体(D2D)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马 D-二聚体(D2D)结合就少，反之结合 就多；反应平衡后，形成固相抗体-生物素化马 D-二聚体(D2D)，再加入酶标记的亲和素，形成 固相抗体-生物素化马 D-二聚体(D2D)-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）。随着马 D-二聚体(D2D)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，对样本中马 D-二聚体(D2D)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	数量	主要成分
校准品	0.5ml/管*6 管	抗原配制的 6 个浓度标准品
包被微孔板	96T/48T	预包被固相抗体
HRP 标记抗体	6mL	HRP 标记的检测抗体
生物素化抗原	6mL	检测抗原
样本稀释液	6mL	磷酸盐缓冲液
底物液 A	6mL	过氧化氢工作液
底物液 B	6mL	TMB 工作液
终止液	6mL	酸性溶液
20×浓缩洗涤液	30mL	含 0.15%Tween20 的 PBS
说明书	1 份	--
自封袋	1 个	--
不干胶	2 片	--

校准品浓度依次为：5000、2500、1250、625、312.5、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.标准规格酶标仪。

2.自动洗板机。

3.振荡器。

4.系列可调节移液器及吸头，一次检测样品较多时，最好用多通道移液器。

试剂盒限制性 仅供科研使用，不得用

于临床诊断。

在试剂盒标示的有效期内使用，过期产品不得使用。

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。

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。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。

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。

注意事项 1)本试剂盒仅供体外研究使用，不用于

临床诊断。

2)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。

3)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。

4)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。

5)消除板底残留的液体和手指印，否则影响 OD 值。

6)底物显色液应呈无色或很浅的颜色。

7)避免试剂和标本的交叉污染以免造成错误结果。

8)在储存和温育时避免强光直接照射。

9)平衡至室温后再打开密封袋以防水滴凝聚在冷板条上。

10)任何反应试剂不能接触漂白溶剂或漂白溶剂所散发的强烈气体。任何漂白成分都会破坏试剂 盒中反应试剂的生物活性。

11)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。

12)请勿使用其他批号或其他来源的试剂混合或替代本试剂盒中的试剂。

13)试验中所用的 EP 管和吸头均为一次性使用，严禁混用。

14)请勿使用过期的试剂。

样品的准备和保存

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.

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

Reagent preparation Before use, all components must be rewarmed for at least 120 minutes to ensure sufficient rewarming to room temperature.

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. 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

1. Move various reagents to room temperature and equilibrate for half an hour. Take the concentrated washing solution, dilute it with distilled water 1:20 according to the number of tests in the current batch, mix well and set aside.

2. Take out the pre-coated plate from the sealed bag, set a blank control well without adding any liquid; set 2 holes for each calibrator, add 50 µl of the corresponding calibrator to each well; add the serum to be tested directly to each of the remaining detection holes. Or 50µl of quality control product.

3. Add 50 µl of biotinylated antigen to all wells except the blank well, mix well, attach sealing film, and incubate at 37°C for 60 minutes. 4. Manual plate washing: discard the liquid in the wells, fill each well with washing solution, let stand for 10 seconds and spin dry, repeat 3 times and pat dry. Wash the plate with a plate washer: select the washing program 3 times and pat dry after washing the plate.

5. Add 50 µl of enzyme-labeled avidin to each well (except the blank control well), mix well, attach a sealing film, and incubate at 37°C for 30 minutes. 6. Manual plate washing: discard the liquid in the wells, fill each well with washing solution, let stand for 10 seconds and spin dry, repeat 3 times and pat dry. Wash the plate with a plate washer: select the washing program 3 times and pat dry after washing the plate.

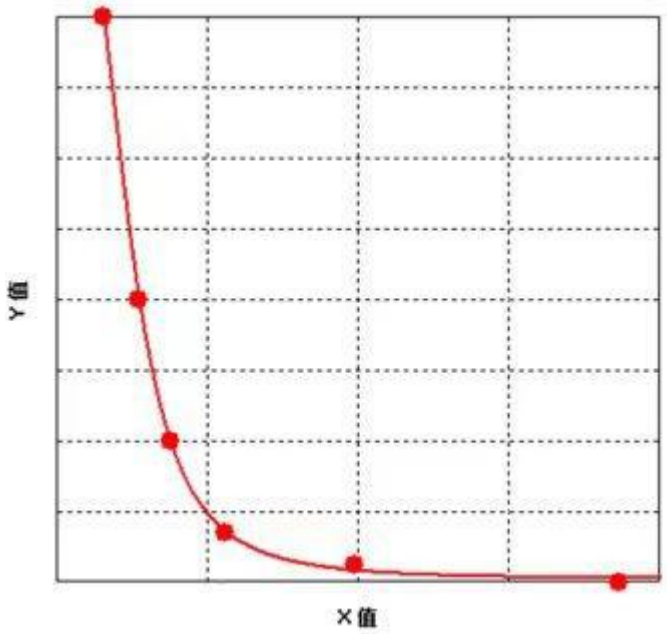
7. Add 50 µl of chromogen A and 50 µl of chromogen B to each well. After shaking and mixing, place at 37°C to develop color in the dark for 15 minutes. Add 50 µl of stop solution to each well.

8. 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.



(Schematic diagram, 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 Causes	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	Improper storage of ELISA kits	Store relevant reagents according to
	Not terminated before reading	Stop solution should be added to