(仅供科研使用，不得用于临床诊断!)

小鼠四旋蛋白 2(TSPAN2)ELISA 试剂盒

使用说明书 产品货号：BY-EM228681 规格：48T/96T 检测范围：6.25 ng/mL- 200 ng/mL。

灵敏度：最低检出剂量小于 1.0 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小鼠四旋蛋白 2(TSPAN2)，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小鼠四旋蛋白 2(TSPAN2) 的浓度。

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 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。

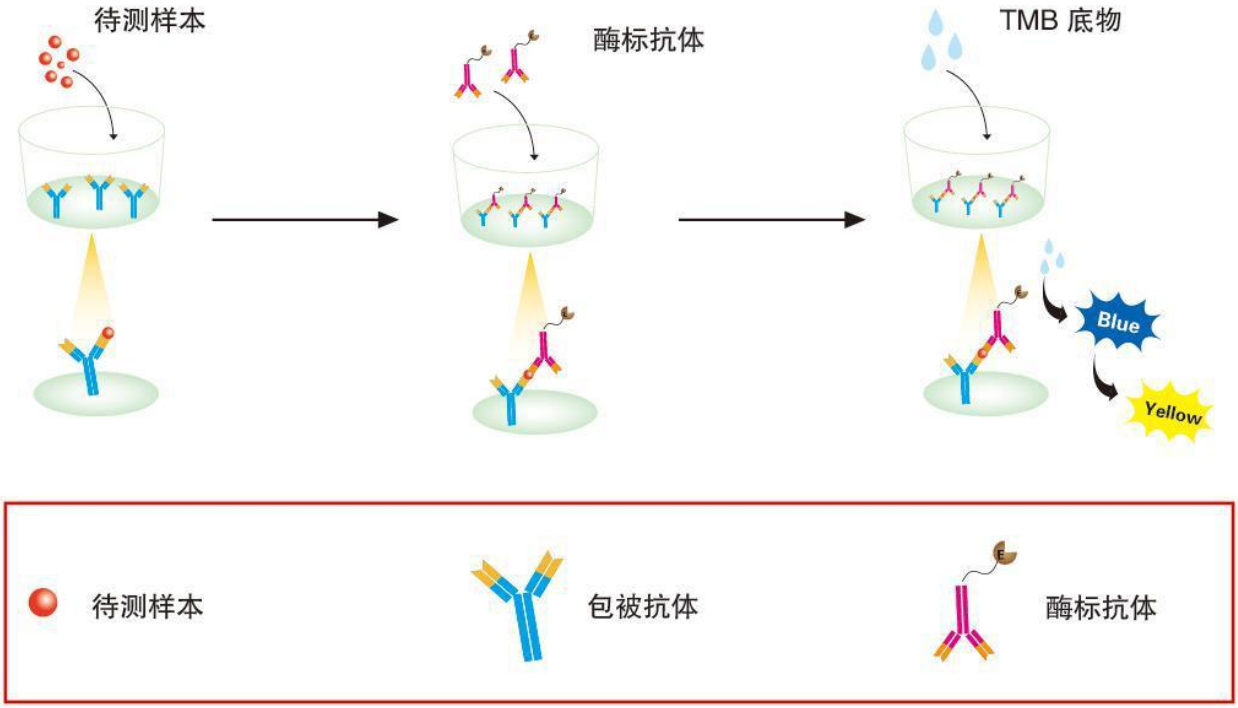
Nanjing BYabscience technology Co.,Ltd

网址：www.byabscience.cn　　官方热线：025-5229-8998　　监督电话：15950492658

实验原理

本试剂盒采用双抗体夹心酶联免疫吸附试验（ELISA）。在预包被抗小鼠四旋蛋白 2(TSPAN2) 抗体（固相抗体）的微孔酶标板中，加入小鼠四旋蛋白 2(TSPAN2) 校准品和待测样本，再加入 HRP 标记的抗小鼠四旋蛋白 2(TSPAN2) 抗体（酶标抗体），经过温育与充分洗涤，去除未结 合的组分，在微孔板固相表面形成固相抗体-抗原-酶标抗体的夹心复合物。加底物 A 和 B，底 物在 HRP 催化下，产生蓝色产物，在终止液（酸性溶液）作用下，最终转化为黄色。在酶标仪 450nm 波长上测定吸光度（OD 值），吸光度（OD 值）与待测样品中小鼠四旋蛋白 2(TSPAN2) 的浓度正相关。拟合校准品曲线，可以计算出样本中小鼠四旋蛋白 2(TSPAN2) 的浓度。

实验原理图



Kit components and storage Unopened kits should be stored 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℃14 days**
Standard product	0.3mL*6 tubes	0.3mL*6 tubes	**2-8℃14 days**
sample diluent	3ml	6ml	2-8℃180 days
HRP labeled antibodies	5ml	10ml	**2-8℃14 days**
Chromogenic substrate A	3ml	6ml	2-8℃180 days
Chromogenic substrate B	3ml	6ml	2-8℃180 days
stop solution	3ml	6ml	2-8℃180 days
20×Lotion	15ml	25ml	2-8℃180 days
sealing film	2 sheets	2 sheets	
manual	1 serving	1 serving	
Ziplock bag	1	1	

The concentrations of calibrators are: 200, 100, 50, 25, 12.5, 6.25 ng/mL.

Note: 1: Please check whether the label and quantity of the reagents in the kit are consistent with the table before use.

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

6) Vortex shaker, microplate shaker

Notes 1) For scientific research use only, not for clinical diagnosis.

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) 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) 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。

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 × g for 5 to 10 minutes, and take the supernatant for detection.

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 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. 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.

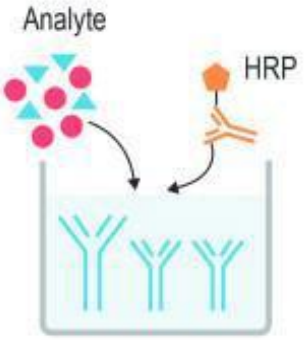
5. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 60 minutes.

6、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

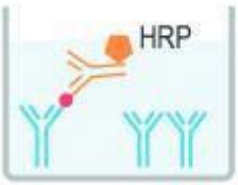
程序，可以提高检测的精度。洗板结束，加底物前，要在干净不掉屑的纸上，充分拍干反应板。（提示：为获得理想的实验结果，必须彻底移除残留液体。洗板完成之后，请立即进行下步操 作，不要让微孔板干燥。）7、将底物 A 和 B 按 1:1 体积充分混合，所有孔中加入底物混合液 100μL。用封板膜盖住反应 板，37℃水浴锅或恒温箱避光孵育 15min。

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

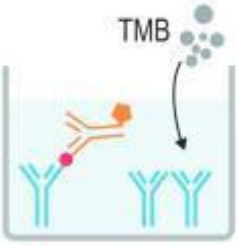
[操作流程图]



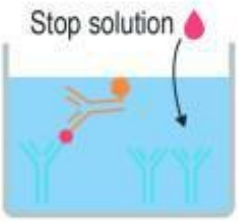
1. 对应板孔中加入50μL标准品工作液或样本后，立即每孔加入100ulHRP酶标抗体工作液，37°C孵育60分钟



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

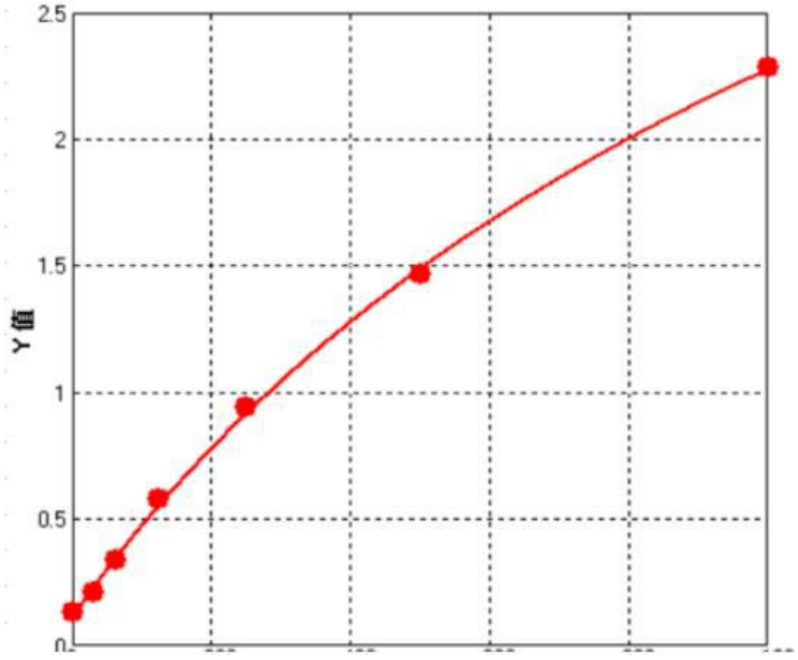


4. 每孔加入50μL终止液

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-pl），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，通过上述方法测的的浓度值，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）

[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
	Equilibration time is too short	Ensure sufficient balancing time
	Incomplete washing	Ensure the washing time and number of washes 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 aspiration and addition process to ensure that all reagents are added in sufficient
	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	Incorrect dosing	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

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 result. 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 that detect the same target substance using different methods, 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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Website: www.byabscience.cn **Official hotline: 025-5229-8998** **Supervision phone number:**