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

# Sheep stromal cell-derived factor 1 (SDF-1) ELISA kit Instructions for use Product number: BY-ES779402 Specifications: 48T/96T Detection range: 150 pg/mL, – 4800 pg/mL.

**Sensitivity: The lowest detectable dose is less than 10 pg/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 sheep stromal cell-derived factor 1 (SDF-1) and has no crossover with structural analogs. Stability: Stored at 2℃-8℃, validity period is 6 months.**

**Purpose: Used to detect the concentration of sheep stromal cell-derived factor 1 (SDF-1) 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.**

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

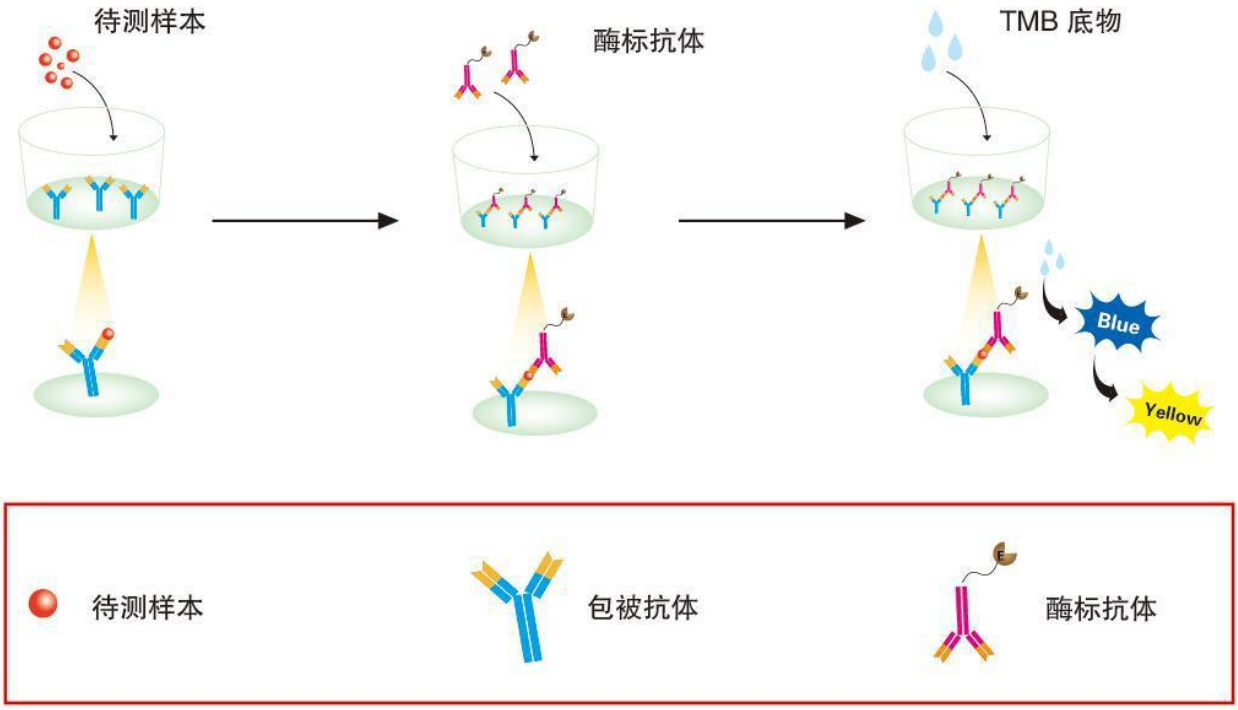
**Nanjing BYabscience technology Co.,Ltd**

**Website: www.byabscience.cn** **Official hotline: 025-5229-8998** **Supervision phone number:**

## Experimental principle

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In the microwell enzyme plate pre-coated with anti-sheep stromal cell-derived factor 1 (SDF-1) antibody (solid-phase antibody), add sheep stromal cell-derived factor 1 (SDF-1) calibrator and the sample to be tested, and then Add HRP-labeled anti-sheep stromal cell-derived factor 1 (SDF-1) antibody (enzyme-labeled antibody), and after incubation and sufficient washing, unbound components are removed, and a solid-phase antibody-antigen is formed on the solid surface of the microplate. -Sandwich complex of enzyme-labeled antibodies. Adding substrates A and B, the substrates are catalyzed by HRP to produce a blue product, which is finally converted to yellow under the action of the stop solution (acidic solution). The absorbance (OD value) was measured at a wavelength of 450 nm using a microplate reader. The absorbance (OD value) was positively correlated with the concentration of sheep stromal cell-derived factor 1 (SDF-1) in the sample to be tested. The concentration of sheep stromal cell-derived factor 1 (SDF-1) in the sample can be calculated by fitting the calibrator curve.

## Experimental schematic diagram

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## 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 |
| 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 | |

校准品浓度依次为：4800 、2400 、1200 、600 、300 、150 pg/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

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) 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。

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.

## 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 × 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 solutions 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.

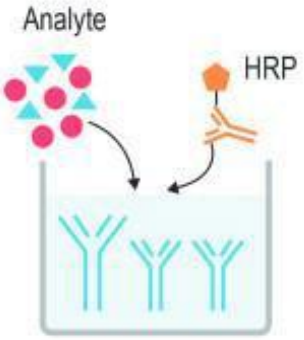
5. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 60 minutes.

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

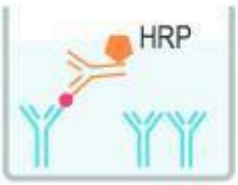
程序，可以提高检测的精度。洗板结束，加底物前，要在干净不掉屑的纸上，充分拍干反应板。（提示：为获得理想的实验结果，必须彻底移除残留液体。洗板完成之后，请立即进行下步操 作，不要让微孔板干燥。）7、将底物 A 和 B 按 1:1 体积充分混合，所有孔中加入底物混合液 100μL。用封板膜盖住反应 板，37℃水浴锅或恒温箱避光孵育 15min。

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）。

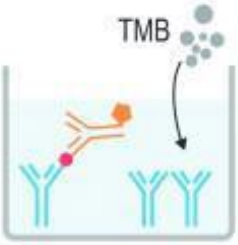
[操作流程图]



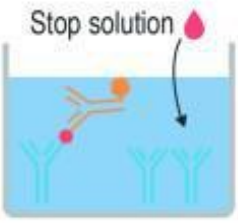

1. 对应板孔中加入50μL标准品工作液或样本后，立即每孔加入100ulHRP酶标抗体工作液，37°C孵育60分钟




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

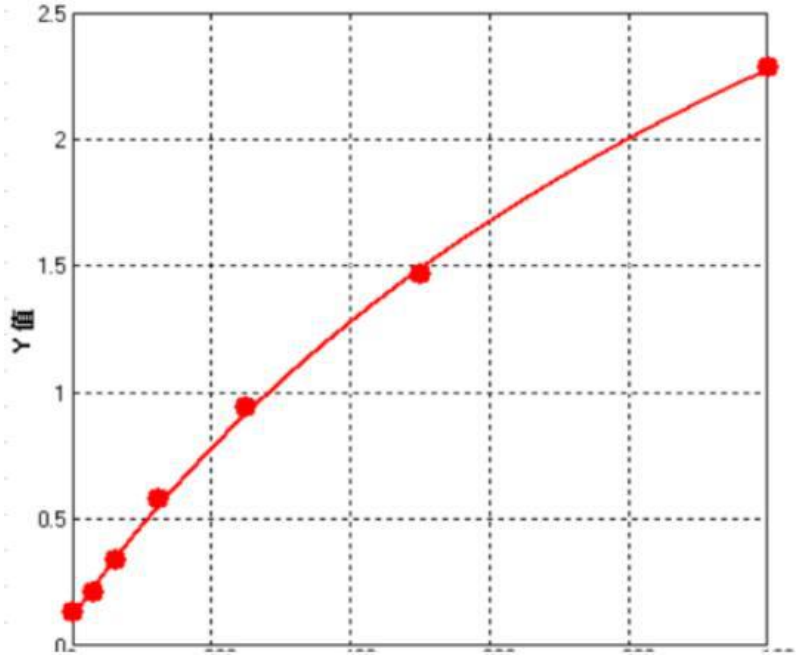



4. 每孔加入50μL终止液

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 |
| | 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 | 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 |

## statement

1. Limited by the existing 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 using different methods to detect the same target, 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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