（本试剂盒仅供体外科研使用，不用于临床诊断）

产品货号：ESP-M0001S

产品规格：96T/96T*5

Elabscience® 小鼠 γ 干扰素（IFN-γ）一体式酶联免疫斑点检测试剂盒使用说明书

Mouse IFN-γ (Interferon Gamma) solid ELISPOT Kit

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：

电话：400-999-2100

邮箱：techsupport@elabscience.cn

网址：www.elabscience.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。

联系时请提供产品批号(见试剂盒标签)，以便我们更高效地为您服务。

用途

该ELISPOT试剂盒用于检测分泌小鼠IFN-γ的细胞频率。
ELISPOT是一种高度特异性的免疫分析方法，用于分析T/B细胞在单细胞水平上的细胞因子和其他可溶性分子的产生和分泌，检测环境与体内环境非常相似，且只需最少的细胞操作。该免疫分析技术的目的是确定细胞在特定刺激下产生细胞因子的频率，并将这种频率与特定治疗或病理状态进行比较。ELISPOT检测是研究T/B细胞功能、自身免疫检测、疫苗开发、器官移植、肿瘤学、传染病、病毒感染监测和治疗等的理想工具。

基本性能

性能	
特异性	该试剂盒可识别/检测小鼠 IFN-γ，且与其它类似物无明显交叉反应
重复性	板内，板间变异系数均<10%

检测原理

本试剂盒是基于双抗夹心法原理，用抗小鼠 IFN-γ 抗体包被于微孔板底部 PVDF 膜上，实验时加入细胞悬液和刺激剂，细胞在培养过程中分泌的小鼠 IFN-γ 会与包被抗体结合。通过洗涤除去细胞和未结合的物质，依次加入生物素化的抗小鼠 IFN-γ 抗体和碱性磷酸酶标记的亲和素。生物素化的抗小鼠 IFN-γ 抗体与结合在包被抗体上的小鼠 IFN-γ 结合、生物素与亲和素特异性结合而形成免疫复合物，游离的成分被洗去。加入显色底物(BCIP/NBT)，BCIP 在碱性磷酸酶的催化下水解并与 NBT 发生反应，形成不溶性的深蓝色至蓝紫色斑点。用专业的 ELISPOT 读板仪软件分析，计算分泌小鼠 IFN-γ 的细胞频率。

试剂盒组成及保存

未拆封的试剂盒可在 2-8℃保存 1 年。试剂盒组分开封后请按照下表中的条件分别保存。

中文名称	规格	开封后保存条件
ELISPOT 板条（不可拆） ELISPOT Plate (Solid)	96T：1 块 96T ELISPOT 板 96T*5：5 块 96T ELISPOT 板	2-8℃，可存放 1 年
浓缩生物素化抗体（100×） Concentrated Biotinylated Detection Ab (100×)	96T：1 支 70μL 96T*5：5 支 70μL	-20℃，可存放 1 年
浓缩 ALP 酶结合物(100×) Concentrated ALP Conjugate(100×)	96T：1 支 70μL 96T*5：5 支 70μL	
生物素化抗体稀释液 Biotinylated Detection Ab Diluent	96T：1 瓶 10mL 96T*5：5 瓶 10mL	2-8℃，可存放 1 年
ALP 酶结合物稀释液 ALP Conjugate Diluent	96T：1 瓶 10mL 96T*5：5 瓶 10mL	
洗涤液 1（即用型、无菌） Wash Buffer 1	96T：2 瓶，25mL/瓶（无菌） 96T*5：10 瓶，25mL/瓶（无菌）	
浓缩洗涤液 2（10×） Concentrated Wash Buffer 2（10×）	96T：2 瓶，25mL/瓶 96T*5：10 瓶，25mL/瓶	
底物溶液（BCIP/NBT） Substrate Reagent	96T：1 瓶 7mL 96T*5：5 瓶 7mL	2-8℃，避光可存放 1 年
产品说明书 Manual	1 份	

说明：所有试剂瓶盖须旋紧以防止蒸发和微生物的污染。
试剂体积以实际发货版说明书为准。相关试剂在分装时会比标签上标明的体积稍多一些，请在使用时量取而非直接倒出。

实验所需自备物品

1. 超净工作台
2. 高精度移液器，EP管及一次性吸头：0.5-10 μL, 2-20 μL, 20-200 μL,200-1000 μL
3. 加样槽
4. 通用离心机
5. 自动细胞计数仪
6. 细胞培养基
7. CO2细胞培养箱
8. 37℃恒温箱
9. 双蒸水或去离子水
10. ELISPOT读板仪

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。处理试剂、血液标本、PBMC、人类细胞系应符合当地的安全条例，例如中华人民共和国卫生行业标准WS 233-2002：“微生物和生物医学实验室生物安全通用准则”。
3) 暂时不用的板条应拆卸后放入备用铝箔袋，按照上述表格中保存条件存放。
4) 请勿重复使用生物素化抗体工作液、酶结合物工作液。未用完的浓缩生物素化抗体(100×)、浓缩ALP酶结合物(100×)、ELISPOT板及其他原液按照上述表格中保存条件存放。
5) 试验中所用的EP管和吸头均为一次性使用，严禁混用。
6) BCIP/NBT底物是有毒性的，可能引起皮肤严重过敏反应，处理该试剂时应小心，始终戴上手套。
7) 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。
8) 请勿使用过期的试剂。
9) 按照实验程序中描述的孵育时间。

检测前准备工作

1. **洗涤液1**：直接使用。提示：从冰箱中取出的浓缩洗涤液1可能有结晶，属于正常现象，可用40℃水浴微加热使结晶完全溶解后使用。（超净工作台操作）
2. **洗涤液2**：将浓缩洗涤液2用双蒸水稀释（1:9）。提示：从冰箱中取出的浓缩洗涤液2可能有结晶，属于正常现象，可用40℃水浴微加热使结晶完全溶解后再配制洗涤液。
3. **生物素化抗体工作液**：实验前计算当次实验所需用量(以50μL/孔计算)，实际配制时应多配制100-200 μL。使用前15分钟，将浓缩生物素化抗体于800×g离心1分钟，以生物素化抗体稀释液将100×浓缩生物素化抗体稀释成1×工作浓度(例如：10 μL浓缩液+990 μL稀释液)。现配现用。
4. **ALP酶结合物工作液**：ALP酶结合物为ALP酶结合亲和素。实验前计算当次实验所需用量(以50μL/孔计算)，实际配制时应多配制100-200 μL。使用前15分钟，将浓缩ALP酶结合物于800×g离心1分钟，以酶结合物稀释液将100×浓缩HRP酶结合物稀释成1×工作浓度(例如：10 μL浓缩液+990μL稀释液)。现配现用。
5. **BCIP/NBT底物溶液**：试剂是即用型的，呈现透明到淡黄色。若有沉淀，使用一次性注射器和0.2μm滤头过滤。

样本准备

1. 在培养基中稀释原代细胞（免疫细胞）或细胞系到合适细胞数，并加入刺激剂（试剂盒"典型数据"中推荐的刺激剂、疫苗、肽池或感染细胞等），所使用细胞的类型、细胞分离方法、刺激方式和孵育时间由研究者决定。
2. 当选择了适合实验的细胞数量后，最后将细胞铺板并刺激它们。除了感兴趣的刺激条件，我们建议每个样本设置三个对照条件（空白对照可以共用）：

对照	试验条件	目的
阳性对照	用经过验证的特异性抗原或多克隆刺激剂培养的细胞。	细胞的功能和实验是否有效。 揭示假阴性结果。
阴性对照	没有刺激的情况下培养的细胞。	自发分泌的细胞数量。 揭示假阳性结果。
空白对照	没有细胞，但有培养基。	试剂或细胞培养基是否产生假斑点。 导致的假阳性结果。

注：

1) 新鲜制备的细胞可以用于ELISPOT实验，冷冻保存的细胞谨慎使用。
2) 本试剂盒适用于悬浮培养的原代细胞和细胞系检测，贴壁细胞尚未进行验证。
3) 在正式实验前，建议做预试验以寻找最佳刺激条件和细胞浓度，以避免斑点汇聚或显色不明显。
4) 建议在ELISPOT正式实验中设置3个复孔测试样本。
5) 足够的刺激需要一定的细胞数量，该实验斑点数和细胞数间并非线性关系。
6) 在ELISPOT板中加入细胞数不宜超过4×105个/孔，通常0.5~2.5×105个细胞/孔用于评估抗原特异性反应。较高的细胞浓度会造成多细胞层，导致斑点形成不良。对于多克隆刺激，可能需要减少细胞数量以避免汇合斑点的形成。

操作步骤

1. 洗板：将 ELISPOT 板从密封包装中取出，用洗涤液 1（200 μl/孔）洗涤 2 次。
2. 加样：甩尽板内液体，每孔加入 100 μL 实验前准备的细胞悬液（每个细胞样本设置阳性对照、阴性对照和空白对照），并盖上培养板板盖。
3. 孵育：将板置于 37℃，5% CO2 的恒温培养箱中，孵育时间从 18~48 小时不等。具体的孵育时间会有所不同，这取决于细胞类型、细胞状态、细胞数量，检测的蛋白质，蛋白质释放动力学以及实验过程中是否包括预刺激等。（注意：在此期间，禁止移动 ELISPOT 板。）
4. 洗板：甩尽板内液体，用洗涤液 2（200 μl/孔）洗涤 5 次。
5. 生物素化抗体：每孔加入 50 μL 生物素化抗体工作液，37℃孵育 2h。
6. 洗板：甩尽板内液体，用洗涤液 2（200 μl/孔）洗涤 5 次。
7. 酶结合物：每孔加入 50 μL 的 ALP 酶结合物工作液，37℃孵育 1h。
8. 洗板：甩尽板内液体，用洗涤液 2（200 μl/孔）洗涤 5 次。
9. 底物：每孔加入 50 μL 的 BCIP/NBT 底物溶液，37℃孵育直至出现明显的斑点，通常为 5-25min。
10. 终止：甩尽板内液体并拆下板框底部的外套，用去离子水/超纯水彻底冲洗 PVDF 膜的上下两面。（板孔内膜上表面每次注满水后甩尽板内液体，并重复这一过程至少 10 次；板孔外膜下表面用去离子水冲洗 3 次）
11. 晾干：在室温下风干(倒扣、避光)。
12. 读数：使用 ELISPOT 读板仪计数斑点。

注：

1) 每次洗板完成后不用拍板。
2) 推荐手动洗板，如使用洗板机洗板，请注意洗板机探头高度，不要接触微孔板底部。
3) 整个实验过程中应避免移液器吸头接触微孔板底部，以防止吸头戳破微孔板底部薄膜。

计算结果

ELISPOT 板通过使用 ELISPOT 读板仪计数斑点来分析。斑点是圆形的，呈典型的"核晕"结构：中心着色较深，边缘略模糊。可以对结果进行定量，例如，计算细胞分泌某蛋白或抗体的频率。

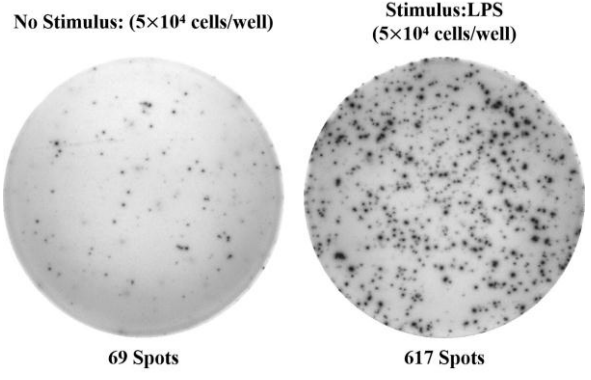
技术资源

如有任何技术问题，请与我司技术支持联系(建议及时对显色结果拍照，保留实验数据、所用板条及未使用的试剂)。

技术支持微信

典型数据

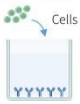
以下数据和斑点图仅供参考。



将小鼠脾细胞(5*10^4 个细胞/孔)在不含或含刺激剂 ConA 的条件下孵育 20h，斑点数为分泌小鼠 IFN-γ 的细胞数量。

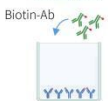
操作一览表

1. 取出ELISPOT板，每孔加入200 μL洗涤液1，洗涤2次



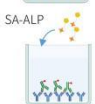
2. 甩尽板内液体，对应板孔中加入100 μL预处理细胞悬液样本，置于37°C，5% CO_2恒温培养箱中孵育18-48 h

3. 甩尽板内液体，每孔加入200 μL洗涤液2，洗涤5次



4. 每孔加入50 μL生物素化抗体工作液，37°C孵育2 h

5. 甩尽板内液体，每孔加入200 μL洗涤液2，洗涤5次



6. 每孔加入50 μL ALP酶结合物工作液，37°C孵育1 h，甩尽板内液体，每孔加入200 μL洗涤液2，洗涤5次

7. 每孔加入50 μL BCIP/NBT底物溶液，37°C孵育5-25 min，直至出现明显的斑点

8. 终止反应（去离子水彻底冲洗板底PVDF膜上下两面），晾干后，使用ELISPOT读板仪计数斑点

声明

1. 限于现有条件及科学技术水平，尚不能对所有原料进行全面的鉴定分析，本产品可能存在一定的质量技术风险。
2. 本试剂盒在研发过程中去除/降低了生物学样本中的一些内源性干扰因素，并非所有可能影响的因素均已去除。
3. 最终的实验结果与试剂的有效性、实验者的相关操作以及当时的实验环境等因素密切相关，本公司只对试剂盒本身负责，不对因使用试剂盒所造成的样本消耗负责，请使用者使用前充分考虑到样本可能的使用量，预留充足的样本。
4. 为了达到好的实验结果，请只使用本公司试剂盒内提供的试剂，不要混用其他制造商的产品，严格按照说明书操作。
5. 即使是相同人员操作也可能在两次独立实验中得到不同的结果，为保证结果的重现性，需要控制实验过程中每一步的操作。
6. 试剂盒发货前会经过严格的质检，然而，因为运输条件、实验设备差异等等因素影响，用户检测结果可能跟出厂数据不一致。不同批次间试剂盒间的差异也可能来自上述原因。
7. 试剂盒仅供研究使用，如将其用于临床诊断或任何其他用途，我公司将不对因此产生的问题负责，亦不承担任何法律责任。

Mouse IFN-γ (Interferon Gamma) solid ELISPOT Kit

Catalog No：ESP-M0001S
Size：96T/96T*5

Intended use

This ELISPOT kit is designed for the detection of the frequency of Mouse IFN-γ secreting cells.
ELISPOT is a highly specific immunological analysis method used to analyze the production and secretion of cytokines and other soluble molecules by T/B cells at the single-cell level. The assay mimics the physiological environment closely and requires minimal cell manipulation. This immunological analysis technique aims to determine the frequency of cells that produce cytokines in response to a given stimulus and compare this frequency with specific treatments or pathological conditions. ELISPOT detection is an ideal tool for studying T/B cell functionality, autoimmune testing, vaccine development, organ transplantation, oncology, infectious diseases, virus infection monitoring, and therapy, among others.

Character

Item	
Specificity	This kit recognizes Mouse IFN-γ in samples. No significant cross-reactivity or interference between Mouse IFN-γ and analogues was observed
Repeatability	Coefficient of variation is < 10%

Test principle

This assay kit uses the Sandwich-ELISA principle. The PVDF membrane at the bottom of the micro ELISPOT plate has been re-coated with anti-Mouse IFN-γ antibodies. During the experiment, cell suspension and stimulants are added first, and IFN-γ secreted by the cells during the incubation process will bind to the coated antibodies. Then wash away the cells, add biotinylated anti-Mouse IFN-γ antibodies and alkaline phosphatase conjugated avidin sequentially. The biotinylated anti-Mouse IFN-γ antibodies will bind specifically to the IFN-γ which has already bounded, then avidin will bind specifically to biotin, together forming the complex. After free components are washed away, add chromogenic substrate (BCIP/NBT), BCIP undergoes hydrolysis under the catalysis of alkaline phosphatase and reacts with NBT, forming insoluble spots with color dark blue to blue-purple. Professional ELISPOT plate reader

software can be used to analyze the result, calculating the frequency of Mouse IFN-γ secreting cells.

Kit components & Storage

An unopened kit can be stored at 2-8℃ for 1 year. After opening, store the items
separately according to the following conditions

Item	Specifications	Storage
ELISPOT Plate (Solid)	96T: 1 plates, 96T 96T*5: 5 plates, 96T	2-8°C, 1 year
Concentrated Biotinylated Detection Ab (100×)	96T: 1 vial 70μL 96T*5: 5 vials 70μL	-20℃, up to expiry date (1 year)
Concentrated ALP Conjugate(100×)	96T: 1 vial 70μL 96T*5: 5 vials 70μL	
Biotinylated Detection Ab Diluent	96T: 1 vial 10mL 96T*5: 5 vials 10mL	2-8°C, 1 year
ALP Conjugate Diluent	96T: 1 vial 10mL 96T*5: 5 vials 10mL	
Wash Buffer 1 (Ready to use, sterile)	96T: 2 vials, 25mL (sterile) 96T*5: 10 vials, 25mL (sterile)	
Concentrated Wash Buffer 2 (10×)	96T: 2 vials, 25mL 96T*5: 10 vials, 25mL	
Substrate Reagent	96T: 1 vial 7mL 96T*5: 5 vials 7mL	2-8°C, protect from light, 1 year
Product Description	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Other supplies required

1. Laminar flow cabinet
2. High-precision pipette, EP tubes and disposable pipette tips: 0.5-10μL, 2-20μL, 20-200μL, 200-1000μL
3. Loading slot
4. General purpose centrifuge
5. Automated cell counter
6. Sterile culture media
7. CO2 cell incubator
8. Incubator capable of maintaining 37°C
9. Deionized or distilled water
10. ELISPOT reader

Note

1) For research use only. Not for use in diagnostic procedures.
2) Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories when handling reagents, blood specimens, PBMC, and human cell lines.
3) Return the unused wells to the foil pouch and store according to the conditions suggested in the above table.
4) Do not reuse the reconstituted biotinylated detection Ab and ALP conjugate working solution. The unspent Detection Ab (100×), ALP Conjugate (100×), ELISPOT Plate and other stock solutions should be stored according to the storage conditions in the table above.
5) All EP tubes and pipette tips used in the experiment are disposable; mixing or reuse is strictly prohibited.
6) BCIP/NBT substrate is toxic and may cause severe skin allergic reactions. Handle the reagents with care, and always wear gloves.
7) Do not mix or substitute reagents from other batches or sources with the reagents included in this assay kit.。
8) The kit should not be used beyond the expiration date on the kit label.
9) Follow the incubation times specified in the experimental procedures.

Reagent preparation

1. **Wash Buffer 1**: Ready to use. Note: concentrated Wash Buffer 1 removed from the refrigerator may contain crystals, which is a normal phenomenon. Note: if crystals have formed inthe concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved. (under ultra-clean table conditions)
2. **Wash Buffer 2**: Dilute Concentrated Wash Buffer 2 with deionized or distilled water at a ratio of 1:9. Note: concentrated Wash Buffer 1 removed from the refrigerator may contain crystals, which is a normal phenomenon. Allow the crystals to completely dissolve by micro-heating in a 40°C water bath (under ultra-clean table conditions) prior to preparation.
3. **Biotinylated Detection Ab Working Solution**: Calculate the required amount before the experiment (50μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 min, then dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent(Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent = 1:99). The working solution should be prepared just before use.
4. **ALP Conjugate working solution**: ALP Conjugate is ALP conjugated avidin. Calculate the required amount before the experiment (50μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated ALP Conjugate at 800×g for 1 min, then dilute the 100× Concentrated ALP Conjugate to 1× working solution with ALP Conjugate Diluent(Concentrated ALP Conjugate: ALP Conjugate Diluent= 1: 99).The working solution should be prepared just before use.
5. **BCIP/NBT Substrate Reagent**: The reagent is ready-to-use and appears clear to pale yellow. If there is any precipitation, filter the reagent using a disposable syringe and 0.2μm membrane filter.

Sample preparation

1. Dilute primary cells (immune cells) or cell lines with the sterile culture medium to an appropriate number, and add a stimulant (stimulants, vaccines, peptide pools, or infected cells, as recommended in the kit's "typical data", etc.). The types of effector and responder cells used, method of cell separation, mode of stimulation, and length of incubation are to be determined by each investigator.
2. Once the suitable cell number for the experiment is determined, plate the cells and stimulate them. In addition to the conditions of interest, we recommend setting three control conditions for each sample (blank control can be shared):

Control	Condition	Purpose
Positive Control	Cells cultured with validated specific antigens or polyclonal stimulants	Assesse cell functionality and experiment efficacy Reveal the false-negative result
Negative Control	Cells cultured without any stimulation	Confirm the number of spontaneously secreting cells Reveal the false-positive result
Blank Control	Culture medium with no cell cultured	Check whether reagents or cell culture medium generate false spots Reveal the false-positive result

Note:

1) Freshly prepared cells are recommended for use in ELISPOT experiments. Frozen cells may be used but should be handled with caution due to potential changes in cell viability or function.
2) This kit is suitable for detection of primary cells and cell lines cultured in suspension, and has not been validated for adherent cells.
3) Before the formal experiment, it is recommended to conduct a pre-test to determine the optimal stimulation condition and cell concentration, to avoid spot aggregation or unclear color development.
4) In the formal ELISPOT experiment, it is advisable to include three replicate wells for each test sample to ensure data reliability and reproducibility.
5) Achieving sufficient stimulation requires a certain number of cells. The relationship between the number of spots generated in the experiment and the number of cultured cells is not linear
6) The number of cells added into the ELISPOT plate should not exceed 4×10^5 cells per well, typically adding $0.5\sim2.5\times10^5$ cells per well for assessing the antigen-specific response. Higher cell concentration may lead to the formation of multilayers, resulting in poor spot formation. For polyclonal stimulation, it might need to reduce cell numbers to avoid the formation of conglomerate spots.

Assay procedure

1. Plate Washing: Remove the ELISPOT plate from its sealed packaging and wash it 2 times with Wash Buffer 1 (200μL/well).
2. Sample Addition: Decant the liquid from each well, add 100μL of the prepared cell suspension to each well (set positive control, negative control, and blank control for each sample), and cover the plate with the plate lid.
3. Incubation: Place the plate in an incubator, 37°C 5% CO2 for 18-48 hours. The specific incubation time depends on cell type, cell status, cell number, the protein being detected, protein release kinetics, and whether the experimental process includes pre-stimulation. (Note: Do not move the ELISPOT plate during the incubation period)
4. Plate Washing: Decant the liquid from each well, Add 200 μL of Wash Buffer 2 to each well, aspirate or decant the solution from each well, and repeat 5 times.
5. Biotinylated Detection Antibody Addition: Add 50μL of Biotinylated Detection Ab working solution to each well and incubate at 37°C for 2 hours.
6. Plate Washing: Decant the liquid from each well, Repeat the wash process for 5 times as conducted in step 4.
7. ALP Conjugate Addition: Add 50μL of ALP Conjugate working solution to each well and incubate at 37°C for 1 hour.
8. Plate Washing: Decant the liquid from each well, Repeat the wash process for 5 times as conducted in step 4.
9. Substrate Addition: Add 50μL of BCIP/NBT Substrate Reagent to each well and incubate at 37°C until evident spots appear, usually for 5-25 minutes.
10. Reaction Termination: Decant the liquid from each well, remove the outer wrap from the bottom of the plate frame, and thoroughly rinse both sides of the PVDF membrane with deionized water. (The inside membrane surface can be washed by filling the well with deionized water and then decanting the liquid, repeat for at least 10 times; directly rinse the outside membrane surface 3 times with deionized water)
11. Drying: Air dry the plate at room temperature. (inverted and light-protected).
12. Reading: Use an ELISPOT reader to count the spots.

Note:

1) No need to slap the plate face-down to thoroughly remove the liquid in the well after washing.
2) Manual plate washing is recommended. If using an automated plate washer, be cautious of the probe height, ensuring it does not touch the bottom of the micro plate.
3) Avoid the contact between the pipette tip and the bottom of the micro plate through the entire experiment to prevent piercing the membrane on the bottom of the micro plate.

Calculation of results

The ELISPOT plate is analyzed by counting the spots using an ELISPOT reader. The spots are circular and have a typical "nuclear halo" structure: the center is darker and the edge is slightly blurred. The results can be quantified, for example, by calculating the frequency of cells secreting a certain protein or antibody.

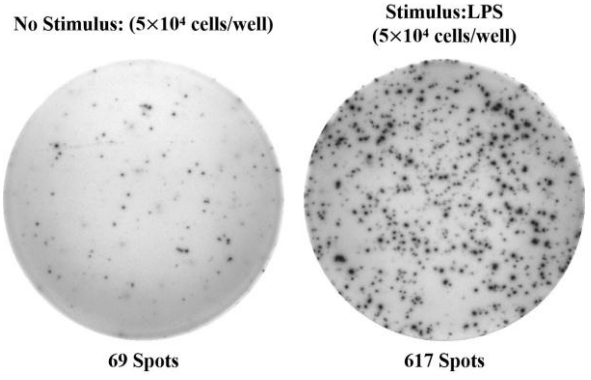
Technology resources

If you have any technical problems, please feel free to contact our technical support (it is recommended to take pictures and save the experimental data in time. Keep the used plate and remaining reagents).

Wechat of technical support

Typical data

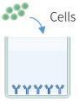
The following data and spot images only for reference



Incubate mouse splenocytes (5*10^4 cells/well) under the condition with or without the stimulus ConA for 20 hours. The spot count represents the number of cells secreting mouse IFN-γ.

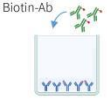
Assay Procedure Summary

1. Remove the ELISPOT plate and wash each well twice with wash buffer 1 (200 µL/well)



2. Decant the liquid, add 100 µL of pre-treated cell suspension sample to each well. Incubate the plate at 37°C in a 5% CO_2 incubator for 18-48 hours

3. Decant the liquid from each well, wash the plate 5 times with wash buffer 2 (200 µL/well)



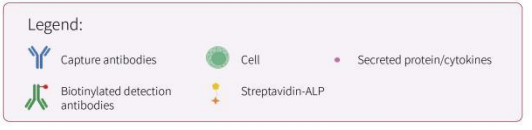
4. Add 50µL of Biotinylated Detection Ab working solution to each well and incubate at 37°C for 2 hours

5. Decant the liquid from each well, wash the plate 5 times with wash buffer 2 (200 µL/well)

6. Add 50µL of ALP Conjugate working solution to each well and incubate at 37°C for 1 hour. Decant the liquid, wash the plate 5 times with wash buffer 2 (200 µL/well)

7. Add 50 µL of BCIP/NBT substrate solution to each well, incubate at 37°C for 5-25 minutes until distinct spots appear

8. Terminate the reaction by thoroughly rinsing the PVDF membrane (both sides) with deionized water. Air-dry and analyze spots with ELISPOT reader



Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.
3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on. We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.
5. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
6. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra-assay variance among kits from different batches might arise from the above reasons too.
7. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.