ELISA 实验报告

一、实验原理

酶联免疫吸附测定（Enzyme-Linked Immunosorbnent Assay，ELISA）是免疫学和分子生物学中广泛使用的实验室技术，由 Eva Engvall 和 Peter Perlmann 于 1971 年首次描述。该测定依赖于抗原-抗体相互作用的原理，并利用酶和比色检测来量化目标分子，主要用于检测和量化生物样品中特定蛋白质、肽、抗体或抗原的存在。ELISA 测定通常应用于各个领域，包括临床诊断、药物研究和生命科学基础研究。

二、实验器材及试剂

1、实验器材

名称	厂家	型号
电子天平	SCALE	ELECTRonic
组织研磨仪	Servicebio	KZ-II
台式高速冷冻离心机	Haier	LX-165T2R
纯水仪	青岛富勒姆科技	FBZ2001-UP-P
漩涡震荡器	Servicebio	MX-F
超低温冰箱	海尔	DW-86L338J
隔热式电热恒温培养箱	恒宇	HGPF-50
全波长酶标仪	赛默飞	Varioskan ALF

2、主要实验耗材

耗材	厂家	货号
1000μL 移液器	大龙	KE0037273
200μL 移液器	大龙	YE3K030591
50μL 移液器	大龙	DS35110
10μL 移液器	大龙	KE0012951
300μL 多道移液器	大龙	KA006318

三、实验方法

1. 样本准备

A. 血清

全血样品于室温放置 1 小时或 2-8℃过夜后于 2-8℃，1000×g 离心 20 分钟，取上清即可检测。上清可存放于-20℃，避免反复冻融。

B. 血浆

抗凝剂推荐使用 EDTA-Na2，样品采集后 30 分钟内于 2-8℃，1000×g 离心 15 分钟，取上清即可检测。-20℃存放，避免反复冻融。。

C. 细胞上清

收集液体后于 2-8℃，1000×g 离心 20 分钟，除去杂质及细胞碎片，取上清检测。-20℃存放，避免反复冻融。

D. 细胞裂解样本

贴壁细胞用冷的 PBS 轻轻清洗，然后用胰蛋白酶消化，1000×g 离心 5 分钟后收集细胞；悬浮细胞可直接离心收集。收集的细胞用冷的 PBS 洗涤 3 次。每 106 个细胞中加入 150-200 μL PBS 重悬(推荐在 PBS 中加入蛋白酶抑制剂;若含量很低可减少

PBS 的体积)并通过反复冻融或超声使细胞破碎。将提取液于 2-8℃，1500×g 离心 10 分钟，取上清检测。-20℃存放，避免反复冻融。

E. 组织裂解样本

用预冷的 PBS (0.01M, pH=7.4)冲洗组织，去除残留血液，称重后将组织剪碎。将剪碎的组织与对应体积的 PBS(一般按 1:9 的重量体积比，比如 1 g 的组织样品对应 9 mL 的 PBS，具体体积可根据实验需要适当调整，并做好记录。推荐在 PBS 中加入蛋白酶抑制剂)加入玻璃匀浆器中，在冰上充分研磨。为了进一步裂解组织细胞，可以对匀浆液进行反复冻融或超声破碎。最后将匀浆液于 2-8℃，5000×g 离心 5-10 分钟，取上清检测。-20℃存放，避免反复冻融。

F. 尿液

收集尿液后，1000x g 离心 20min，取上清，-20℃或者-80℃存放，避免反复冻融。

G. 母乳

收集样本后，10000x g 离心 15min，取澄清部分，浑浊液再次离心取澄清，收集澄清部分，-20℃存放，避免反复冻融。

2. 试剂准备

A. 标准品

每次实验新溶解一瓶标准品。建议标准品和样本都进行复孔检测。

每瓶冻干品，按说明书推荐的稀释液加对应体积，做标记“sd1”，以此作为标准曲线的起始浓度。溶解后的冻干品需要在 30min 内用完。

在“sd2”“sd3”“sd4”“sd5”“sd6”“sd7”EP 管中各加入 0.5mL 对应的样本稀释液，取 0.5mL“sd1”加入“sd2”中进行梯度稀释。梯度稀释时需充分混匀。

B. 检测抗体

检测抗体（100x）浓缩液：按稀释比例 1：100 稀释，稀释前根据预先计算好的

每次实验所需的总量配置（100μL/孔），实际配置时应配置（110μL/孔）。如 10μL 检测抗体加 990μL 抗体稀释液的比例配置，轻轻混匀。

C. HRP 标记抗体/HRP 标记链霉亲和素

HRP 标记抗体/HRP 标记链霉亲和素（100x）浓缩液：按稀释比例 1：100 稀释，稀释前根据预先计算好的每次实验所需的总量配置（100μL/孔），实际配置时应配置（110μL/孔）。如 10μLHRP 标记抗体加 990μL 抗体稀释液的比例配置，轻轻混匀。

D. 洗涤液

浓缩洗涤液（20x）使用前需平衡室温。（如果洗涤液（20x）有晶体析出，37℃加热至晶体全部溶解。）取 30mL 浓缩洗涤液（20x），加入 570mL 超纯水或去离子水，得到洗涤液（1x）

3. 操作步骤

1) 加样，分别设空白孔、标准孔、待测样品孔。空白孔加样品稀释液 100μL，标准孔加标准品 100μL 或待测样品孔加 100μL，注意不要有气泡，加样时将样品加于酶标板孔底部，尽量不触及孔壁。确保上样不间断，5-10min 上完样本。
2) 酶标板上覆膜，37℃孵育 120min。
3) 洗涤，揭开封板膜，弃液体，毛巾或者滤纸将板内残留液体拍出，用洗涤液（1x）洗涤板条 4 次，每孔 350-400μL，最后一次洗涤后，确保板内无残留液体，避免毛巾或者滤纸纤维进入板内，板条也不能长时间放室温导致干透。
4) 每孔加入 100μL（1x）检测抗体，盖上封板膜，37℃孵育 1h。重复步骤 3
5) 每孔加入 100μL（1x）HRP 标记抗体/HRP 标记链霉亲和素，盖上封板膜，37℃孵育 40min。重复步骤 3
6) 显色：每孔加入 TMB 显色液 100μL，37℃避光显色 15-20min（如果颜色过浅，可适当延长显色时间，不超过 30min）。
7) 终止：每孔加终止液 100μL，此时蓝色变为黄色。终止液的加入顺序应与 TMB

显色液的加入顺序一致。

8) 读数：以 630nm 为校准波长，用酶标仪在 450nm 波长测量各孔的光密度（OD 值）。在加终止液后 5min 内进行读数。

4. 数据分析

每个标准品和样本的 OD 值需减去零孔的 OD 值，如设置复孔，则应取其平均值。以标准品的浓度为横坐标，OD 值为纵坐标，使用 ELISA Calc 回归拟合计算程序进行四参数拟合。根据样品的 OD 值由标准曲线推算出相应的浓度，再乘以稀释倍数。

ELISA Report

Experimental principle

Enzym-linked Immunosorbnent Assay (ELISA) is a widely used laboratory technique in immunology and molecular biology, first described by Eva Engvall and Peter Perlmann in 1971. The assay relies on the principle of antigen-antibody interactions and utilizes enzyme and colorimetric assays to quantify target molecules, primarily for detecting and quantifying the presence of specific proteins, peptides, antibodies, or antigens in biological samples. ELISA assays are commonly used in various fields, including clinical diagnosis, drug research, and basic research in life sciences.

Laboratory equipment and reagents

Laboratory equipment

Equipment	Manufacturers	Model
Electronic balance	SCALE	ELECTRonic
Tissue Grinder	Servicebio	KZ-II
High speed refrigerated centrifuge	Haier	LX-165T2R
Laboratory pure water system	Zhiang Instrument (Shanghai) Co., Ltd	Clever-S
Vortex shaker	Servicebio	MX-F
Ultra-low temperature refrigerator	Haier	DW-86L338J
Thermal insulation electric temperature incubator	NanTong Hengyu Scientific instrument co., LTD	HGPF-50

Full wavelength enzyme labeling instrument	MolecμLar Devices	Spectramax M2

Laboratory consumables

Consumables	Manufacturers	Catlog
0-1000μL Adjustable-Volume Pipettor	Dragon	KE0037273
0-200μL Adjustable-Volume Pipettor	Dragon	YE3K030591
0-50μL Adjustable-Volume Pipettor	Dragon	DS35110
0-10μL Adjustable-Volume Pipettor	Dragon	KE0012951
0-300μL Adjustable-Volume multichannel pipettor	Dragon	KA006318

Experimental method

Preservation of samples

A. serum

The whole blood samples were left at room temperature for 1 hour or 2-8℃ overnight before centrifugation for 20 minutes at 1000 g, removing the supernatant to be detected. The supernatant can be stored at-20℃ to avoid repeated freezing and thawing.

B. blood plasma

EDTA-NA 2 is recommended for anticoagulants, centrifugation at 2-8℃ for 15 minutes at 1000 g within 30 minutes after sample collection and detection with the supernatant.-20℃ storage, avoid repeated freezing and thawing.

C. The cell supernatant

After liquid collection, the samples was centrifuged at 2-8℃ for 20 minutes at 1000 g to remove impurities and cell debris and remove the supernatant for detection.-20℃

storage, avoid repeated freezing and thawing.

D. Cell lysed samples

Adherent cells were then gently washed with cold PBS, then digested with trypsin and centrifuged at 1000 g for 5 minutes; cells in suspension could be collected by direct centrifugation. The ted cells were washed three times with cold PBS. Resuspend 150-200 μL PBS per 106 cells (protease inhibitor to PBS recommended; reduce PBS volume if low) and crush cells by repeated freezing-thawing or sonication. The extract was centrifuged at 2 – 8℃ at 1500 g for 10 min and the supernatant was removed for detection.-20℃ storage, avoid repeated freezing and thawing.

E. Tissue cleavage samples

The tissue was washed with pre-cooled PBS (0.01M, pH=7.4) to remove the residual blood, weighed and then chopped the tissue. Cut the tissue to the corresponding volume of PBS (generally according to the volume ratio of 1:9 by weight, such as 1 g of tissue sample corresponding to 9 mL PBS, the specific volume can be adjusted according to the needs of the experiment, and recorded. Protease inhibitor in PBS) to the glass homogenizer and thoroughly ground on ice. For further lysis of histiological cells, the homogenate can be subjected to repeated freeze-thaw or sonication. Finally, the homogenate was centrifuged at 2 – 8℃ at 5000 g for 5 – 10 minutes and the supernatant was removed for detection.-20℃ storage, avoid repeated freezing and thawing.

F. urine

After urine collection, the sample was centrifuged at 1000 xg for 20min and the supernatant was removed and stored at-20℃ or-80℃ to avoid repeated freezing and thawing.

G. breast milk

After sample collection, the samples were centrifuged at 10000 xg for 15min, the

clarified part was removed, the turbid fluid was centrifuged again for clarification, and the clarified part was collected and stored at-20℃ to avoid repeated freezing and thawing.

Preservation of Reagent

A. standard substance

A new bottle of the standard was dissolved in each experiment. It is recommended for both standards and samples.

For each lyophilized bottle, add the corresponding volume as recommended by the instructions and mark "sd 1" as the starting concentration of the standard curve. The dissolved lyophilized products should be used up within 30min.

0.5 mL of corresponding sample dilution was added to each of the "sd 2" "sd 3" "sd 4" "sd 6" "sd 7" EP tubes and 0.5 mL of "sd 1" was added to "sd 2" for gradient dilution. The gradient dilution should be fully mixed.

B. Detection of antibodies

Test antibody (100x) concentration: 1:100 dilution. Before dilution, the total amount (100 μ L / well), and the actual configuration shall be configured (110 μ L / well). Like 10 μ L detection antibody plus 990 μ L antibody dilution ratio configuration, gently mix well.

C. The HRP-labeled antibody / HRP was labeled with streptavidin

HRP-labeled antibody / HRP-labeled streptavidin (100x) concentrate: diluted 1:100 before dilution (100 μ L / well), and actual configuration (110 μ L / well). As the proportional configuration of 10 μL HRP labeled antibody plus 990 μ L antibody dilution, gently mix well.

D. Washing solution

Concentrated detergent (20x) shall be balanced at room temperature before use.(If the washing solution (20x) is crystals, heat 37℃ until all the crystals are dissolved.) Take 30

mL of concentrated washing solution (20x) and add 570 mL of ultrapure water or deionized water to obtain the washing solution (1x)

Experimental procedure

1. Sample addition: Set up blank hole, standard hole and sample hole to be tested respectively. Add 100 μ L of sample dilution in blank hole, 100 μ L of standard well or 100 μ L of sample hole to be tested, note no bubbles, add the sample to the bottom of the hole of the microplate when sampling, and try not to touch the hole wall. Ensure the sample is uninterrupted, 5-10min.

2. Microplate was coated with film and incubated at 37℃ for 120min.

3. Wash, open the sealing plate film, discard the liquid, towel or filter paper to pat the residual liquid in the board, wash the board with washing liquid (1x) 4 times, 350-400 μ L per well, after the last washing, ensure that there is no residual liquid in the board, avoid towel or filter paper fiber into the board, the board can not be put at room temperature for a long time to dry.

4. Add 100 μ L (1x) to each well, covered with the plate membrane, and incubated at 37℃ for 1h. Repeat step 3

5. 100 μ L (1x) HRP labeled antibody / HRP labeled streptavidin was added to each well, covered with the sealed plate membrane, and incubated at 37℃ for 40min. Repeat step 3

6. Color development: add 100 μ L of TMB color development solution to each well, 37℃ of light avoidance and color development for 15-20min (if the color is too light, the color development time can be extended appropriately, no more than 30min).

7. Termination: Add 100 μ L of termination solution to each well, when the blue becomes yellow. The addition order of the termination solution should be consistent with that of the TMB color development solution.

8. Reading: Measure the optical density (OD) of each hole at 450nm at 630nm with a microplate reader. Read reading was performed within 5min after adding the termination solution.

Data analysis

The OD value of each standard and sample shall subtract the OD value of zero hole, and take the average value if set. With the concentration of the standard product as the horizontal coordinate and OD value as the vertical coordinate, ELISA Calc regression fitting calculation program was used for four-parameter fitting. The corresponding concentration was calculated from the standard curve based on the OD of the sample and multiplied by the dilution.