ELISA 实验报告

一、实验器材及试剂

1. 实验器材

名称	厂家	型号
立式冷藏陈列柜	星星	LSC-316C
卧式冷冻箱	美菱	BCD-318AT
电子天平	梅特勒-托利多仪器（上海）有限公司	ME203E/02
高速组织研磨仪	Servicebio	KZ-II
台式高速冷冻离心机	大龙	D3024R
全自动洗板机	雷杜生命科技有限公司	RT-3500
电热鼓风干燥箱	Labotery	GEL-70
实验室超纯水机	艾肯水精灵	AK-RO-C2
涡旋混合器	Servicebio	MX-F
酶标检测仪	雷杜生命科技有限公司	RT-6100

2. 主要实验耗材

耗材	厂家	货号
1.5ml 离心管	Servicebio	EP-150-M
2ml 离心管	Servicebio	EP-200-M
1250ul 吸头	Servicebio	TP-1250
200ul 吸头	Servicebio	TP-200
10ul 吸头	Servicebio	TP-10
1000ul 移液器	大龙	KE0037273
200ul 移液器	大龙	YE3K030591
50ul 移液器	大龙	DS35110

10ul 移液器	大龙	KE0012951
300ul 多道移液器	大龙	KA006318

二、样本的采集与保存

1. 动植物组织样本(干冰运输)

准确称取动物组织重量按重量(mg)：体积(ul)=1:9 的比例加入 9 倍体积的匀浆介质(推荐 0.86%或 0.9%的生理盐水)，冰水浴条件下，机械匀浆，制备成 10%的匀浆液，2500~3000 转/分钟，离心 10 分钟，取上清液进行测定。

2. 血清样本(干冰运输)

全血标本请于室温放置 2 小时或 4℃过夜后于 2-8℃ 3000 转/分离心 15min，取上清即可立即检测;或进行分装，并将标本放于-20℃或-80℃保存，但应避免反复冻融。解冻后的样本应再次离心，然后检测。

3. 血浆样本(干冰运输)

可用 EDTA 或肝素作为抗凝剂，标本采集后 30 分钟内于 2-8℃3000 转/分离心 15min，取上清即可立即检测;或进行分装，并将标本放于-20℃或-80℃保存，但应避免反复冻融。解冻后的样本应再次离心，然后检测。

4. 细胞样本

贴壁细胞：将细胞用 PBS 冲洗 2 次后，用细胞刮将细胞小心刮下来，将培养液 3000 转/分，离心 10min。弃上清留细胞沉淀，干冰运输。

悬浮细胞：将培养液 3000 转/分，离心 10min。弃上清留细胞沉淀，干冰运输。

细胞上清：标本于 2-8℃，3000 转/分离心 15min 取上清，上清立即用于实验，或分装后于-20℃或-80℃保存。避免反复冻融。

三、ELISA 实验步骤

按试剂盒说明书进行操作，不同指标操作不一样。具体以订购的试剂盒说明书为准（见附件）。基本步骤如下：

1. 包被：用碳酸盐包被缓冲液将抗体稀释至蛋白质含量为 1-10μg / ml。在聚苯乙烯酶标板的每孔加 100μl，4℃过夜。次日，弃去孔内溶液，用洗涤缓冲液洗 3 次，每次 3min。（一般商品化的试剂盒中已包被好抗体，此步骤可省略）

2. **封闭**：每孔加入 200ul 封闭液，37℃孵育 1-2 h。
3. **洗涤**：小心揭掉封板膜，放入洗板机，洗涤 3-5 遍。也可以手动洗板：弃去液体，每孔加入 300ul 洗液，浸泡 1-2min，在吸水纸上拍干，重复 3-5 遍。（一般商品化的试剂盒中已包被好抗体，前三个步骤可省略）
4. **加样**：加适当稀释的待检样品 100μl 于上述已包被的反应孔中。(同时做空白孔，倍比稀释的标准品孔，有条件可加做阴性对照孔及阳性对照孔作为质控点)。
5. **温育**：用封板膜封板后置 37℃孵育 1-2 h。
6. **洗涤**：同步骤 3。
7. **加抗体**：于各孔中加稀释好的生物素化抗体工作液 100 μl。
8. **温育**：用封板膜封板后置 37℃孵育 1 h。
9. **洗涤**：同步骤 3。
10. **加酶结合物**：于各孔中加稀释好的酶结合物工作液 100 μl。
11. **温育**：用封板膜封板后置 37℃避光孵育 30 min。
12. **洗涤**：同步骤 3。
13. **加显色底物**：于各孔中加入 TMB 底物溶液 100 μl，37℃避光反应 10～30min，直到倍比稀释的标准品孔出现明显的颜色梯度为止。
14. **终止反应**：于各反应孔中加入 2M 硫酸 100 μl，颜色由蓝色变为黄色。
15. **结果测定**：10min 内，在酶标仪上，于 450nm 处，以空白对照孔调零后测各孔 OD 值。

四、ELISA 结果计算

根据标准品的浓度和 OD 值做标准曲线，然后根据标准曲线方程计算出样本浓度。

结果以 ECXEL 形式发送

ELISA Report

1. Apparatus and Reagents

1.1 Apparatus

Equipment	Manufacturers	Model
Vertical refrigerated display cabinet	XingXing	LSC-316C
Horizontal freezer	MeiLing	BCD-318AT
Electronic balance	Mettler-Toledo	ME203E/02
High Speed Tissue Grinder	Servicebio	KZ-II
High speed refrigerated centrifuge	Dragon	D3024R
Automatic Plate Washer	Rayto	RT-3500
Electrothermal blast drying oven	Labotery	GEL-70
Ultrapure Water Polishing System	Aiken water Elf	AK-RO-C2
Vortex Mixer	Servicebio	MX-F
Enzyme label detector	Rayto	RT-6100

1.2 Laboratory consumables

Equipment	Manufacturers	Model
1.5ml eppendorf tubes	Servicebio	EP-150-M
2ml eppendorf tubes	Servicebio	EP-200-M
1250ul micropipette tip	Servicebio	TP-1250
200ul micropipette tip	Servicebio	TP-200
10ul micropipette tip	Servicebio	TP-10
0-1000ul Adjustable-Volume Pipettor	Dragon	KE0037273
0-200ul Adjustable-Volume Pipettor	Dragon	YE3K030591
0-50ul Adjustable-Volume Pipettor	Dragon	DS35110

2. Collection and preservation of samples

2.1 Animal and plant tissue samples (dry ice transport)
Accurately weigh the weight of animal tissue and add 9 times of the volume of homogenizing medium (0.86% or 0.9% normal saline is recommended) according to the proportion of weight (mg): Volume (UL): 1:9. Under the condition of ice water bath, mechanical homogenization is prepared into 10% homogenizing solution, 2500-3000 RPM, centrifugation for 10 minutes, and take the supernatant for determination..

2.2 Serum samples (dry ice transport)
The blood sample should be placed at room temperature for 2 hours or 4 ℃ overnight, then turned at 2-8 ℃ 3000 / centrifuged for 15 minutes, and the supernatant can be detected immediately; or it should be repackaged and stored at - 20 ℃ or - 80 ℃, but repeated freezing and thawing should be avoided. The thawed sample should be centrifuged again and tested.

2.3 Plasma sample (dry ice transport)
Heparin can be used as anticoagulant. Within 30 minutes after collection, the sample can be immediately detected at 2-8 ℃ for 3000 revolutions / centrifuged for 15 minutes; or it can be separately packed and stored at - 20 ℃ or - 80 ℃, but repeated freezing and thawing should be avoided. The thawed sample should be centrifuged again and tested.

2.4 Cell sample
Adherent cells: after washing the cells twice with PBS, scrape the cells carefully with cell scrape, and centrifugate the culture medium at 3000 rpm for 10min. Discard the supernatant and leave the cells to precipitate and transport with drikold.
Suspension cells: the culture medium was centrifuged for 10 minutes at 3000 rpm. Discard the supernatant and leave the cells to precipitate and transport with drikold.
Cell supernatant: the supernatant of the sample was centrifugated at 2-8 ℃, 3000 RPM for 15 minutes, and it was immediately used for the experiment, or stored at - 20 ℃ or - 80 ℃ after separation. Avoid repeated freezing and thawing.

3. Assay Procedure

According to the instructions of the kit, the operation of different indicators has difference. The details are subject to the instructions of the ordered Kit (see the attachment). The basic steps are as follows:

3.1 **Coating:** dilute the antibody with carbonate coating buffer to 1-10 μg /ml protein content. Add 100 μl to each well of polystyrene enzyme plate, and stay overnight at 4 ℃. The next day, discard the solution and wash it with washing buffer for 3 times, each time for 3 minutes. (the antibody has been packed in the general commercial kit, this step can be omitted)

3.2 **Blocking**: add 200ul of blocking buffer into each hole and incubate at 37 ℃ for 1-2 H.

3.3 **Washing**: carefully remove the blocking buffer and put it into the washing machine for 3-5 times. You can also wash the board manually: discard the liquid, add 300ul of wash buffer into each well, soak for 1-2min, pat dry on the absorbent paper, and repeat for 3-5 times. (the first three steps can be omitted)

3.4 **Sample addition**: add 100 μ l of the sample to be tested to the coated reaction pore. (at the same time, blank well and standard well with multiple dilutions can be made. If possible, negative control and positive control can be added as quality control points).

3.5 **Warm Incubation**: seal the plate with plate sealer and incubate at 37 ℃ for 1-2 H.

3.6 **Washing**: synchronous step 3.

3.7 **Add antibody**: add 100 μ l diluted biotinylated antibody working solution to each well.

3.8 **Warm Incubation**: seal the plate with sealing membrane and incubate at 37 ℃ for 1 h.

3.9 **Washing**: synchronous step 3.

3.10 **Adding enzyme conjugates**: add 100 μ l diluted enzyme conjugates working solution to each well.

3.11 **Warm Incubation**: use the plate sealer to seal the plate and then incubate at 37 ℃ in dark for 30 min.

3.12 **Washing**: synchronous step 3.

3.13 **Add the chromogenic substrate**: add 100 μl of TMB substrate solution into each well, react in the dark at 37 ℃ for 10-30min, until there is an obvious color gradient in the times diluted standard pore.

3.14 **Stop reaction**: add 2 m sulfuric acid 100 μ l into each reaction well, and the color changes from blue to yellow.

3.15 **Results**: within 10 minutes, on the enzyme labeling instrument, at 450 nm, the O.D. of each well was measured after the blank control pore was zeroed.

4. Calculation of Results

The standard curve was made according to the concentration and O.D. of the standard, and then the sample concentration was calculated according to the standard curve equation.

The results are sent as ECXEL