
本试剂盒只能用于科学研究，不得用于医学诊断

裸鼠白细胞介素 6 (IL-6) ELISA 科研试剂盒

使用说明书

货号: HG046128

检测原理

试剂盒采用双抗体一步夹心法酶联免疫吸附试验 (ELISA)。往预先包被裸鼠白细胞介素6 (IL-6) 抗体的包被微孔中, 依次加入标本、标准品、HRP 标记的检测抗体, 经过温育并彻底洗涤。用底物TMB显色, TMB在过氧化物酶的催化下转化成蓝色, 并在酸的作用下转化成最终的黄色。颜色的深浅和样品中的裸鼠白细胞介素6 (IL-6) 呈正相关。用酶标仪在450nm 波长下测定吸光度 (OD 值), 计算样品浓度。

样品收集、处理及保存方法

液体样本

- 1、血清: 用无菌管收集, 室温血液自然凝固 120 分钟或 2-8℃下过夜, 在 2-8℃条件下离心 20 分钟 (3000 转/分), 小心仔细收集上清即可检测, 或存储在-20℃或-80℃备用。但应避免反复冻融。
- 2、血浆: 应根据标本的要求选择 EDTA 或肝素钠作为抗凝剂, 混合均匀后 30 分钟内, 2-8℃条件离心 20 分钟 (3000 转/分), 小心仔细收集上清即可检测, 或存储在-20℃或-80℃备用。但应避免反复冻融。
- 3、尿液、胸腹水、脑脊液、唾液: 用无菌管收集, 2-8℃条件离心 20 分钟 (3000 转/分)。小心仔细收集上清, 或存储在-20℃或-80℃备用。但应避免反复冻融。
- 4、细胞培养上清: 用无菌管收集, 2-8℃条件离心 20 分钟 (3000 转/分), 小心仔细收集上清, 或存储在-20℃或-80℃备用。但应避免反复冻融。

固体样本

1、动物组织样本：用预冷的 PBS (0.01M, pH=7.4)冲洗组织，去除残留血液，称重后将组织剪碎成小块。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 1g 的组织样品对应 9mL 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（低温下）充分研磨。或者在组织研磨仪中进行研磨，如果需要进一步裂解组织细胞，可以对匀浆液进行超声破碎，最后将匀浆液 2-8℃条件下 5000×g 离心 10 分钟，取上清进行检测。对于植物组织或其他组织样本，在玻璃匀浆器或组织研磨仪中匀浆不彻底的话，应在液氮中进行充分研磨。

2、细胞样本：

A、动物细胞：对于贴壁细胞，用适量预冷的 PBS 轻轻清洗细胞，并用胰蛋白酶消化分离细胞。以 1000×g 离心 5 分钟收集细胞（悬浮细胞可直接离心收集）。弃上清，用冷 PBS 清洗细胞 3 次。在浓度为 1×10^7 个细胞/mL 的冷 PBS 中重新悬浮细胞。用超声破碎仪充分破碎细胞，以使细胞破坏并放出细胞内成份。2-8℃条件离心 20 分钟（3000 转/分），随后小心仔细取上清液进行检测。

B、植物细胞：用 pH7.2-7.4 的 PBS 稀释细胞悬液，使细胞浓度达到 100 万/ml 左右，置于冰盒上，用超声破碎仪，充分破碎细胞。2-8℃条件离心 20 分钟（3000 转/分），小心仔细收集上清进行检测。

3、咽拭子：加入 2ml 的 pH7.2-7.4 左右的 PBS，溶解咽拭子头部，摇匀，用镊子取出咽拭子并挤干液体，2-8℃条件离心 20 分钟左右（2000-3000 转/分），仔细收集上清。分装一份待检测，其余冷冻备用，保存过程中如有沉淀形成，上样检测前应再次离心。样本的要求标本采集后尽早进行提取，提取后应尽快进行实验。若不能马上进行试验，样品在 2-8℃可保存时应在 6 天内使用，否则必须在 -20℃(≤1 个月)或 -80℃(≤2 个月)保

存，应避免反复冻融。保存过程中如有沉淀形成，上样检测前应再次离心。不能检测含 NaN₃ 的样品，因 NaN₃ 抑制辣根过氧化物酶的（HRP）活性。

自备物品

1.酶标仪（450nm）

2.高精度加样器及枪头：0.5-10 μ L、2-20 μ L、20-200 μ L、200-1000 μ L

3.37 $^{\circ}$ C恒温箱

操作注意事项

1. 试剂盒保存在 2-8 $^{\circ}$ C，使用前室温平衡 20 分钟。从冰箱取出的浓缩洗涤液会有结晶，这属于正常现象，水浴加热使结晶完全溶解后再使用。
2. 实验中不用的板条应立即放回自封袋中，密封（低温干燥）保存。
3. 浓度为 0 的 S0 号标准品即可视为阴性对照或者空白；按照说明书操作时样本已经稀释 5 倍，最终结果乘以 5 才是样本实际浓度。
4. 严格按照说明书中标明的时间、加液量及顺序进行温育操作。
5. 所有液体组分使用前充分摇匀。
6. 暂时用不到的酶标板条取下来放进铝箔袋备用，2-8 $^{\circ}$ C保存。建议尽快使用。

试剂盒组成

| 名称 | 96 孔配置 | 48 孔配置 | 备注 |
|-------------------|--------------------|--------------------|----------|
| 微孔酶标板 | 8 孔 \times 12 条 | 8 孔 \times 6 条 | 无 |
| 标准品 | 0.3mL \times 6 管 | 0.3mL \times 6 管 | 无 |
| 样本稀释液 | 6mL | 3mL | 无 |
| 检测抗体-HRP | 10mL | 5mL | 无 |
| 20 \times 洗涤缓冲液 | 25mL | 15mL | 按说明书进行稀释 |
| 底物 | 12mL | 6mL | 无 |
| 终止液 | 6mL | 3mL | 无 |
| 封板膜 | 2 张 | 2 张 | 无 |
| 说明书 | 1 份 | 1 份 | 无 |

注：标准品（S0-S5）浓度依次为：0、2、4、8、16、32 pg/mL

试剂的准备

20×洗涤缓冲液的稀释：蒸馏水按 1: 20 稀释，即 1 份的 20×洗涤缓冲液加 19 份的蒸馏水。

洗板方法

1. 手工洗板：甩尽孔内液体，每孔加满洗涤液，静置 1min 后甩尽孔内液体，在吸水纸上拍干，如此洗板 5 次。
2. 自动洗板机：每孔注入洗液 350 μ L，浸泡 1min，洗板 5 次。

操作步骤

1. 从室温平衡 20min 后的铝箔袋中取出所需板条，剩余板条用自封袋密封放回 4 $^{\circ}$ C。
2. 设置标准品孔和样本孔，标准品孔各加不同浓度的标准品 50 μ L。
3. 样本孔先加待测样本 10 μ L，再加样本稀释液 40 μ L，空白孔不加。
4. 除空白孔外，标准品孔和样本孔中每孔加入辣根过氧化物酶（HRP）标记的检测抗体 100 μ L，用封板膜封住反应孔，37 $^{\circ}$ C 水浴锅或恒温箱温育 60min。
5. 弃去液体，吸水纸上拍干，每孔加满洗涤液，静置 1min，甩去洗涤液，吸水纸上拍干，如此重复洗板 5 次（也可用洗板机洗板）。
6. 每孔加入底物 100 μ L，37 $^{\circ}$ C 避光孵育 15min。
7. 每孔加入终止液 50 μ L，15min 内，在 450nm 波长处测定各孔的 OD 值。

结果判断

绘制标准曲线：在 Excel 工作表中，以标准品浓度作横坐标，对应 OD 值作纵坐标，绘制出标准品线性回归曲线，按曲线方程计算各样本浓度值。



试剂盒性能

4. 准确性：标准品线性回归与预期浓度相关系数 R 值，大于等于 0.9900。
5. 灵敏度：最低检测浓度小于 1 pg/mL。
6. 检测范围：1 - 32 pg/mL。
7. 特异性：不与其它可溶性结构类似物交叉反应。
8. 重复性：板内、板间变异系数均小于 15%。
9. 贮藏：2-8°C，避光防潮保存。
10. 有效期：6 个月。

免责声明

1. 试剂盒仅供研究使用，不得用于临床实验或人体实验，否则所产生的一切后果，由实验者承担，本公司概不负责。
2. 严格按照说明书操作，实验者违反说明书操作，后果由实验者承担。

**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

Nude mouse IL-6 ELISA Kit instruction

Intended use

This IL-6 ELISA kit is intended Laboratory for Research use only and is not for use in diagnostic or therapeutic procedures. The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of IL-6 in the sample, this IL-6 ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus IL-6 concentration. The concentration of IL-6 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Sample collection and storages

Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at approximately 3000×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 30 minutes at 3000×g at 2-8°C within 30 minutes of collection. Store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.



Note: The samples should be centrifuged adequately and no hemolysis or granule was allowed.

Materials required but not supplied

1. Standard microplate reader(450nm).
2. Precision pipettes and Disposable pipette tips.
3. 37 °C incubator.

Precautions

1. Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched

for optimal performance. Use only the reagents supplied by manufacturer.

2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
3. Mix all reagents before using.

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C).

Materials supplied

| Name | 96 determinations | 48 determinations |
|------------------------|-------------------|-------------------|
| Microelisa stripplate | 8*12strips | 8*6strips |
| Standard | 0.3ml*6tubes | 0.3ml*6tubes |
| Sample Diluent | 6.0ml | 3.0ml |
| HRP-Conjugate reagent | 10.0ml | 5.0ml |
| 20X Wash solution | 25ml | 15ml |
| Chromogen Solution | 12.0ml | 6.0ml |
| Stop Solution | 6.0ml | 3.0ml |
| Closure plate membrane | 2 | 2 |
| User manual | 1 | 1 |

Note: Standard (S0 → S5) concentration was followed by:0,2,4,8,16,32 pg/mL.

Reagent preparation

20×wash solution:Dilute with Distilled or deionized water 1:20.

Assay procedure

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.
2. Add standard: Set Standard wells, testing sample wells. Add standard 50µl to standard well.
3. Add Sample: Add testing sample 10µl then add 40µl of Sample Diluent to testing sample well; Blank well doesn't add anything.
4. Add 100µl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Solution (400µl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.

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6. Add chromogen solution 100 μ l to each well. Gently mix and incubate for 15 minutes at 37°C. **Protect from light.**
 7. Add 50 μ l Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
 - 8 Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

Calculation of results

1. This standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.
2. First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the zero standard before result interpretation. Construct the standard curve using graph paper or statistical software.
3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.
4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.
5. The sensitivity by this assay is 1 pg/mL.
6. Standard curve.



Storage: 2-8°C.
Validity: six months.

FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS! PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

黄页生物
Nuang Guan