（本试剂盒仅供体外研究使用，不用于临床诊断!）

产品货号：E-UNEL-M0070

产品规格：96T*5/96T*15

# Elabscience®未包被小鼠白介素 6(IL-6)酶联免疫吸附测定试剂盒使用说明书

Uncoated Mouse IL-6(Interleukin 6) ELISA 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：


| | |
|---|---|
| 销售部电话 | 027-87879180，027-87854967 |
| 技术部电话 | 027-87526315 |
| 电子邮箱（销售） | Perry@elabscience.cn |
| 电子邮箱（技术） | 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

该试剂盒用于体外定量检测小鼠血清、血浆样本中IL-6浓度。

## 试剂盒组成及保存

| 中文名称 | 规格 | 稀释推荐/质量 | 保存条件 |
|---|---|---|---|
| 小鼠 IL-6 ELISA 预制酶标板<br>Mouse IL-6 Micro ELISA pre-Plate | 96T*5：5 块 96T 酶标板<br>96T*15：15 块 96T 酶标板 | / | -20℃，可存放 6 个月 |
| 小鼠 IL-6 包被抗体<br>Mouse IL-6 Capture Ab | 96T*5：1 支 120μL<br>96T*15：1 支 350μL | 1/500-1/1000 | |
| 小鼠 IL-6 生物素化抗体<br>Mouse IL-6 Biotinylated Detection Ab | 96T*5：1 支 120μL<br>96T*15：1 支 350μL | 1/500-1/1000 | |
| 小鼠 IL-6 冻干标准品<br>Mouse IL-6 Reference Standard | 96T*5：1 支<br>96T*15：3 支 | 20000 pg | |
| 小鼠 IL-6 HRP 酶结合物<br>Mouse IL-6 HRP Conjugate | 96T*5：1 支 120μL<br>96T*15：1 支 350μL | 1/500-1/1000 | -20℃(避光)<br>可存放 6 个月 |
| 产品说明书<br>Manual | 1 份 | / | |
| 质检报告<br>Certificate of Analysis | 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。

## 其他所需试剂

- **ELISA辅助组分试剂盒** (Ancillary Reagent Kit，货号：**E-ELIR-K001**)：包含完成96T*5规格ELISA实验的全套辅助试剂。
- 或有其他实验需求，可单独购买以下辅助试剂产品：

| 名称 | 规格 | 货号 |
|---|---|---|
| 高吸附性 ELISA 包被液(5×)<br>ELISA Plate Coating Buffer(5×) | 14 mL | E-ELIR-002 |
| 即用型 ELISA 封闭液<br>ELISA Plate Blocking Buffer | 100 mL | E-ELIR-003 |
| 通用型夹心法 ELISA 洗涤液(25×)<br>Wash Buffer for Sandwich-ELISA(25×) | 55 mL | E-ELIR-004 |
| 终止液(5×)<br>Stop Solution(5×) | 5 mL | E-ELIR-012 |
| 即用型 HRP 酶结合物稀释液<br>HRP-conjugate Diluent | 60 mL | E-ELIR-008 |
| 即用型生物素化抗体稀释液<br>Biotinylated Antibody Diluent | 60 mL | E-ELIR-010 |
| 通用型样品稀释液<br>Sample Diluent | 60 mL | E-ELIR-011 |
| 单组份 TMB 显色液<br>One-component TMB Substrate | 50 mL | E-IR-R201 |
| 封板覆膜<br>Plate Sealer | 25 张 | |
| 产品说明书<br>Manual | 1 份 | |

- 或自行配制指标通用性辅助试剂。(注：下列配方均为各试剂的基础组分，可根据实验需求和实验结果对配方进行优化)
  - 包被液：1xCBS
  - 封闭液：1xPBS，保护性蛋白
  - 洗涤液：3%Tris
  - 样本稀释液：1xPBS，保护性蛋白
  - 抗体/酶结合物稀释液：1xPBS，保护性蛋白
  - 终止液：5%硫酸

## 试验所需自备物品

1. 酶标仪(450 nm波长滤光片)
2. 高精度移液器，EP管及一次性吸头：0.5-10μL, 2-20μL, 20-200μL, 200-1000μL
3. 37℃恒温箱
4. 双蒸水或去离子水
5. 吸水纸
6. 加样槽

## 样品收集方法

(具体处理方法可参考官网：http://www.elabscience.cn/List-detail-241.html)

1. **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。
2. **血浆**：抗凝剂推荐使用EDTA-$Na_2$，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。

**样本处理相关试剂推荐：**

10×EDTA抗凝剂（货号：E-EL-SR003)

## 注意事项

### ■ 试剂盒注意事项

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。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。
3) 刚开启的酶标板孔中可能会有少许水样物质，此为正常现象，不会对实验结果造成任何影响。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) 请勿重复使用已稀释过的标准品、生物素化抗体工作液、酶结合物工作液。未用完的包被抗体浓缩液、生物素化抗体浓缩液、HRP酶结合物浓缩液、酶标板及其他原液按照上述表格中保存条件存放。
5) 检测使用的酶标仪需要安装能检测450±2 nm波长的滤光片，光密度范围在0-3.5之间。建议使用时提前15分钟预热。
6) 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。
7) 试验中所用的EP管和吸头均为一次性使用，严禁混用。
8) 请勿使用过期的试剂。

### ■ 样品注意事项

1) 收集血液的试管应为一次性无内毒素试管。避免使用溶血，高血脂样品。
2) 样品收集后若在1周内进行检测可保存于2-8℃，若不能及时检测，请按一次使用量分装，冻存于-20℃(1个月内检测)，或-80℃(3个月内检测)，避免反复冻融。在检测前，冷冻过的样本应缓慢地融化并离心除去冻融过程产生的沉淀物。室温混匀后使用。
3) **试剂盒检测范围不等同于样本中待测物的浓度范围**。如果样品中待测物浓度过高或过低，请对样本做适当的稀释或浓缩。
4) 若所检样本不在说明书所列样本之中，建议做预实验验证其检测有效性。若使用化学裂解液制备样本，由于引入某些化学物质会导致ELISA测值出现偏差。
5) 某些重组蛋白可能与试剂盒中捕获或检测抗体不匹配而出现不能检测的情况。

## 检测前准备工作

1. 使用前将待使用的试剂平衡至室温(18-25℃)。如果试剂盒需分多次使用，请仅取出本次实验所需的酶标板条和试剂，剩余板条和试剂需按照指定条件保存。

**2. 酶标板：**

**a)** 使用**高吸附性ELISA包被液（5×）**将**包被抗体**稀释至工作浓度(推荐稀释1/500-1/1000倍)。

**b)** 取出**ELISA预制酶标板**，每孔加入100 μL**包被抗体工作液**，酶标板加上覆膜, 2-8℃孵育过夜。

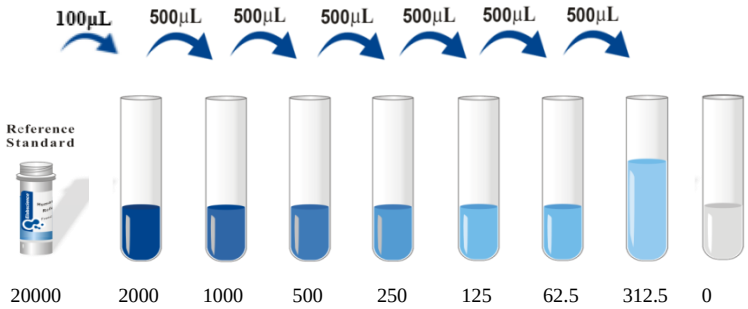
**c)** 甩尽孔内液体，不用洗涤。每孔加入200 μL**即用型ELISA封闭液**，酶标板加上覆膜，37℃温育1小时。

d) 甩尽孔内液体，不用洗涤，可立即开展后续加样操作。或将酶标板置于37℃干燥30分钟，干燥后的酶标板可用干燥剂密封后，-20℃存放6个月。

3. **标准品工作液：**

a) 将**标准品**于10000×g离心1分钟，加入**通用型样品稀释液**1 mL至冻干标准品中，旋紧管盖，静置10分钟，上下颠倒数次，待其充分溶解后，轻轻混匀，避免起泡，配成20000 pg/mL的标准品工作液(或加入1 mL**通用型样品稀释液**后，静置1-2分钟，用低速涡旋仪充分混匀。可通过低速离心去除涡旋过程中产生的气泡)。然后根据需要进行稀释。建议配制成以下浓度：2000、1000、500、250、125、62.5、312.5、0 pg/mL。

b) 倍比稀释方法：先取1支EP管，从20000 pg/mL的标准品工作液中吸取900 μL到第一支EP管中混匀配成2000 pg/mL的标准品工作液。
再取6支EP管，每管中加入500 μL通用型样品稀释液，从2000 pg/mL的标准品工作液中吸取500 μL到第一支EP管中混匀配成1000 pg/mL的标准品工作液，按此步骤往后依次吸取混匀。如下页图示。提示：最后一管直接作为空白孔，不需要再从倒数第二管中吸取液体。倍比稀释的标准品工作液需要现配现用。




4. **生物素化抗体工作液：**实验前计算当次实验所需用量(以100 μL/孔计算)，实际配制时应多配制100-200 μL。使用前15分钟，将**生物素化抗体**于800×g离心1分钟，使用**即用型生物素化抗体稀释液**将**生物素化抗体**稀释成工作浓度(推荐稀释1/500-1/1000倍)。现配现用。
5. **HRP酶结合物工作液：**HRP酶结合物为HRP酶结合亲和素。实验前计算当次实验所需用量(以100 μL/孔计算)，实际配制时应多配制100-200 μL。使用前15分钟，将**HRP酶结合物**于800×g离心1分钟，使用**即用型酶结合物稀释液**将**HRP酶结合物**稀释成工作浓度(推荐稀释1/500-1/1000倍)。现配现用。
6. **洗涤液：**将**通用型夹心法ELISA洗涤液用**双蒸水稀释(1:24)。提示：从冰箱中取出的浓缩洗涤液可能有结晶，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。当日使用。

## 稀释方案指导

**稀释 100 倍**：一步稀释。取 5 μL 样本/浓缩液到 495 μL 稀释液内，做 100 倍稀释；
**稀释 1000 倍**：两步稀释。取 5 μL 样本/浓缩液到 95 μL 稀释液内，做 20 倍稀释，再取 5 μL 20 倍稀释的样本/浓缩液到 245 μL 稀释液内，做 50 倍稀释，总共稀释 1000 倍；
**稀释 100000 倍**：三步稀释。取 5 μL 样本/浓缩液到 195 μL 稀释液内，做 40 倍稀释，再取 5 μL 40 倍稀释的样本/浓缩液到 245 μL 稀释液内，做 50 倍稀释，最后取 5 μL 2000 倍稀释的样本/浓缩液到 245 μL 稀释液内，做 50 倍稀释，总共稀释 100000 倍；
每步稀释时取液量不少于 **3 μL**，稀释倍数不超过 **100 倍**。每步稀释都需混合均匀，避免起泡。

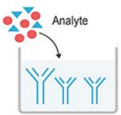
## 通用操作步骤

1. 分别设定标准孔、空白孔和样本孔。标准孔加入100 μL倍比稀释的标准品，空白孔加入100 μL通用型样品稀释液，其余孔加入100 μL待测样本(建议所有的待检样本和标准品在检测中设立复孔；建议通过预实验或咨询技术支持确定待检样本的稀释倍数)。给酶标板覆膜，37℃孵育90分钟。提示：加样时将样品加于酶标板底部，尽量不触及孔壁，轻轻晃动混匀，避免产生气泡。加样时间宜控制在10分钟内。
2. 甩尽孔内液体，不用洗涤。每个孔中加入生物素化抗体工作液100 μL，酶标板加上覆膜，37℃温育1小时。
3. 甩尽孔内液体，在洁净的吸水纸上拍干。每孔加洗涤液350 μL，浸泡1分钟，吸去或甩掉酶标板内的液体，拍干。重复此洗板步骤3次。提示：此处与其他洗板步骤都可使用洗板机(参考北京拓普DEM-3型洗板机参数设置：2点吸，每孔加入洗涤液350 μL，振板5秒，吸液0.5秒)。洗板完成后请立即进行下步操作，不要让微孔板干燥。
4. 每孔加HRP酶结合物工作液100 μL，酶标板加上覆膜，37℃温育30分钟。
5. 甩尽孔内液体，洗板5次，方法同步骤3。
6. 每孔加即用型底物溶液(TMB)90 μL，酶标板加上覆膜，37℃避光孵育15分钟左右。提示：根据实际显色情况酌情缩短或延长，但不可超过30分钟。当标准孔出现明显梯度时(前4个显色孔出现明显蓝色梯度)，即可终止。提前15分钟打开酶标仪预热。
7. 每孔加即用型终止液50 μL，终止反应。提示：终止液的加入顺序应尽量与底物溶液的加入顺序相同。
8. 立即用酶标仪在450 nm波长测量各孔的光密度(OD值)。

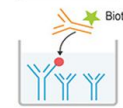
# 操作一览表



1.1 取出ELISA预制酶标板，每孔加入100μL包被抗体工作液，2-8℃孵育过夜
1.2 甩尽孔内液体，每孔加入200μL ELISA封闭液，37℃孵育60分钟



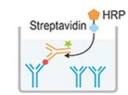

2. 甩尽孔内液体，对应板孔中加入100μL标准品工作液或样本，37℃孵育90分钟



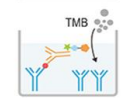

3. 弃掉板内液体后，立即加入100μL生物素化抗体工作液，37℃孵育60分钟



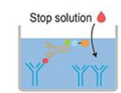

4. 弃掉板内液体，洗板3次




5. 每孔加入100μL HRP酶结合物工作液，37℃孵育30分钟，弃掉板内液体，洗板5次




6. 每孔加入90μL底物溶液，37℃孵育15分钟左右




7. 每孔加入50μL终止液



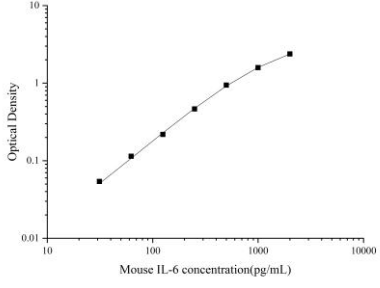
8. 立即在450nm波长下读数，处理数据

## 结果判断

1. 计算标准品和样本复孔的平均OD值并减去空白孔的OD值作为校正值。以浓度为横坐标，OD值为纵坐标，在双对数坐标轴上拟合四参数逻辑函数的标准曲线。
2. 若样品OD值高于标准曲线上限，应适当稀释后重测并在计算样本浓度时乘以相应的稀释倍数。

## 典型数据

以下数据和曲线仅供参考，实验者需根据自己的实验建立标准曲线。

| pg/mL | OD | 校正 OD | 标曲 |
|---|---|---|---|
| 2000 | 2.45 | 2.38 |  |
| 1000 | 1.655 | 1.585 | |
| 500 | 1.014 | 0.944 | |
| 250 | 0.534 | 0.464 | |
| 125 | 0.288 | 0.218 | |
| 62.5 | 0.184 | 0.114 | |
| 312.5 | 0.124 | 0.054 | |
| 0 | 0.07 | - | |



## 性能

■ 特异性

本试剂盒可检测样本中的小鼠IL-6，且与其它类似物无明显交叉反应。

## 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：

| 问题描述 | 可能原因 | 相应对策 |
| --- | --- | --- |
| 标准曲线梯度差 | 核心试剂比例不合适 | 调整捕获抗体、检测抗体比例及蛋白浓度 |
| | 吸液或加液不准 | 检查移液器及吸头 |
| | 标准品稀释不正确 | 溶解标准品时稍微旋转瓶身，轻轻混匀使粉末完全溶解 |
| | 洗涤不完全 | 保证洗涤时间和洗涤次数及每孔的加液量 |
| 显色很弱或无色 | 孵育时间太短 | 保证充足的孵育时间 |
| | 实验温度不正确 | 使用推荐的实验温度 |
| | 试剂体积不够或漏加 | 检查吸液及加液过程，保证所有试剂按顺序足量添加 |
| | 稀释不正确 | |
| | 酶标记物失活或底物失效 | 混合酶结合物和底物，通过迅速显色来检查判断 |
| | 捕获抗体或检测抗体比例不合适 | 调整包被抗体或检测抗体的比例 |
| 读数数值低 | 酶标仪设置不正确 | 在酶标仪上检查波长及滤光片设置 |
| | | 提前打开酶标仪预热 |
| 变异系数大 | 加液不正确 | 检查加液情况 |
| | 出现失误或等待时间过久 | 减少实验失误及等待时间 |
| 背景值高 | 抗体或HRP酶的工作浓度过高 | 使用推荐的稀释倍数，或根据结果自行调整抗体或酶的比例 |
| | 酶标板洗涤不完全 | 保证每步清洗完全；如果用自动洗板机，请检查所有的出口是否有堵塞；是否使用试剂盒配备的洗涤液 |
| | 洗液有污染 | 配制新鲜的洗液 |
| | 缓冲体系不合适 | 更换其他缓冲液体系 |
| 灵敏度低 | 试剂盒保存不当 | 按说明书要求保存相关试剂 |
| | 读数前未终止 | OD 读数前应在每孔中加入终止液 |

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# Uncoated Mouse IL-6(Interleukin 6) ELISA Kit

Catalog No: E-UNEL-M0070
Size: 96T*5/96T*15

## Intended use

This ELISA kit applies to the in vitro quantitative determination of Mouse IL-6 concentrations in serum, plasma.

## Kit components & Storage

| Item | Specifications | Dilution | Storage |
|---|---|---|---|
| Mouse IL-6 Micro ELISA pre-Plate | 96T*5: 5 plates, 96T<br>96T*15: 15plates, 96T | / | -20℃, 6 months |
| Mouse IL-6 Capture Ab | 96T*5: 1 vial, 120μL<br>96T*15: 1 vial, 350μL | 1/500-1/1000 | |
| Mouse IL-6 Biotinylated Detection Ab | 96T*5: 1 vial, 120μL<br>96T*15: 1 vial, 350μL | 1/500-1/1000 | |
| Mouse IL-6 Reference Standard | 96T*5: 1 vial<br>96T*15: 3 vial | 20000 pg | |
| Mouse IL-6 HRP Conjugate | 96T*5: 1 vial, 120μL<br>96T*15: 1 vial, 350μL | 1/500-1/1000 | -20 ℃ (Protect from light), 6 months |
| Product Description | 1 copy | / | |
| Certificate of Analysis | 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 required reagents

- **Ancillary Reagent Kit** (Cat No. E-ELIR-K001): The kit contains a full set of ancillary reagents to complete the 96T*5 ELISA assay.
- Or if there are other experimental requirements, the following auxiliary reagent products may be purchased separately:

| Item | Specifications | Catalog No. |
|---|---|---|
| ELISA Plate Coating Buffer(5×) | 14 mL | E-ELIR-002 |
| ELISA Plate Blocking Buffer | 100 mL | E-ELIR-003 |
| Wash Buffer for Sandwich-ELISA(25×) | 55 mL | E-ELIR-004 |
| Stop Solution(5×) | 5 mL | E-ELIR-012 |
| HRP-conjugate Diluent | 60 mL | E-ELIR-008 |
| Biotinylated Antibody Diluent | 60 mL | E-ELIR-010 |
| Sample Diluent | 60 mL | E-ELIR-011 |
| One-component TMB Substrate | 50 mL | E-IR-R201 |
| Plate Sealer | 25 pieced | |
| Product Description | 1 copy | |

- Or refer to the following formula to prepare each universal reagent.

  (Note: The following formula only contains the basic component information of each reagent, which can be optimized according to the experimental requirements and results)

  - Coating Buffer: 1×CBS
  - Blocking Buffer: 1×PBS, Protective protein
  - Wash Buffer: 3% Tris
  - Standard & Sample Diluent: 1×PBS，Protective protein
  - Antibody & HRP conjugate Diluent: 1×PBS，Protective protein
  - Stop Solution: 5% sulfuric acid

## Other supplies required

Microplate reader with 450 nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
Incubator capable of maintaining 37℃
Deionized or distilled water
Absorbent paper
Loading slot

## Sample collection

(More detailed information please view our website: http://www.elabscience.cn/List–detail-241.html)

**Serum:** Allow samples to clot for 1 hour at room temperature or overnight at 2-8℃before centrifugation for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

**Plasma:** Collect plasma using EDTA-$Na_2$ as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8℃ within 30 min of collection. Collect the supernatant to carry out the assay.

**Recommended reagents for sample preparation:**

10×EDTA Anticoagulant (Cat No. E-EL-SR003)

## Note

### ■ Note for kit

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, especially when detecting blood samples or other bodily fluids.
3) A freshly opened ELISA plate may appear a water-like substance, which is normal and will not have any impact on the experimental results. 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 standard, biotinylated detection Ab working solution, HRP conjugate working solution. The unspent undiluted biotinylated detection Ab and other stock solutions should be stored according to the storage conditions in the above table.
5) The microplate reader should be able to be installed with a filter that can detect the wave length at 450±2 nm. The optical density should be within 0-3.5. Follow the Instructions of the Microplate Reader for set-up and preheat it for 15 min before OD measurement.
6) Do not mix or substitute reagents with those from other lots or sources.
7) Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.
8) The kit should not be used beyond the expiration date on the kit label.

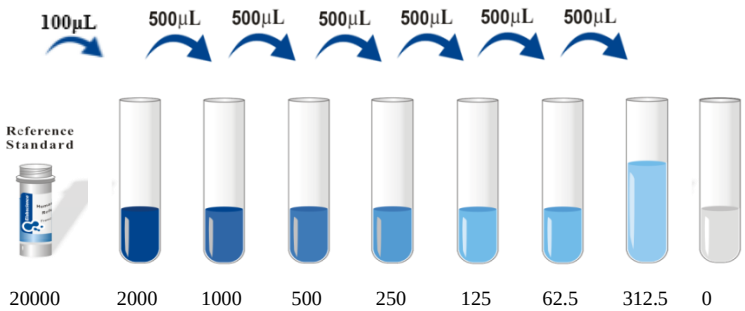
### ■ Note for sample

1) Tubes for blood collection should be disposable and be non-endotoxin. Samples with high hemolysis or much lipid are not suitable for ELISA assay.
2) Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.
3) Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
4) If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity. If a lysis buffer is used to prepare samples, there is a possibility of causing a deviation due to the introduced chemical substance.
5) Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

## Reagent preparation

1. Bring all reagents to room temperature (18-25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Micro ELISA Plate:**
   a) The capture antibody was diluted to the working concentration using the ELISA Plate Coating Buffer(1×) (1/500-1/1000 fold dilution is recommended).
   b) Take out the Micro ELISA pre-Plate, add 100 μL of capture antibody working solution to each well. Cover the plate with the sealer provided in the kit. Incubate overnight at 2-8℃.
   c) Decant the liquid from each well, do not wash. Add 300 μL of ELISA Plate Blocking Buffer to each well. Cover the plate with the sealer. Incubate for 1 hour at 37℃.
   d) Decant the liquid from each well, do not wash, and the plate is ready for sample addition. Or the plate was dried at 37℃ for 30 min. The dried plate can be stored at -20℃ for 6 months after sealing with desiccant.
3. **Standard working solution:**
   a) Centrifuge the standard at 10,000×g for 1 min. Add 1 mL of Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 20000 pg/mL (or add 1 mL of Sample Diluent, let it stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles generated during vortex could be removed by centrifuging at a relatively low speed). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 2000, 1000, 500, 250, 125, 62.5, 312.5, 0 pg/mL.
   b) Dilution method: Take 1 EP tubes, pipette 900 μL of the 20000 pg/mL working solution to the first tube and mix up to produce a 2000 pg/mL working solution. Take 6 EP tubes, add 500 μL of Sample Diluent to each tube. Pipette 500 μL of the 2000 pg/mL working solution to the first tube and mix up to produce a 1000 pg/mL working solution. Pipette 500 μL of the solution from the former tube into the latter one according to this step. The illustration on the next page is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube. Gradient diluted standard working solution should be prepared just before use.
4. **Biotinylated Detection Ab working solution:**
   Calculate the required amount before the experiment (100 μL/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 Biotinylated Detection

Ab to working solution with Biotinylated Antibody Diluent (1/500-1/1000 fold dilution is recommended). The working solution should be prepared just before use.




5. **HRP Conjugate working solution:** HRP Conjugate is HRP conjugated avidin. Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the HRP Conjugate at 800×g for 1 min, then dilute the HRP Conjugate to working solution with HRP Conjugate Diluent (1/500-1/1000 fold dilution is recommended). The working solution should be prepared just before use.
6. **Wash Buffer:** Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved. For same day use only.

## Dilution Method

For 100 fold dilution: One-step dilution. Add 5 μL sample/concentrate to 495 μL diluent to yield 100 fold dilution.

For 1000 fold dilution: Two-step dilution. Add 5 μL sample/concentrate to 95 μL diluent to yield 20 fold dilution, then add 5 μL 20 fold diluted sample/concentrate to 245 μL diluent, after this, the neat sample has been diluted at 1000 fold successfully.

For 100000 fold dilution: Three-step dilution. Add 5 μL sample/concentrate to 195 μL diluent to yield 40 fold dilution, then add 5 μL 40 fold diluted sample/concentrate to 245 μL diluent to yield 50 fold dilution, and finally add 5 μL 2000 fold diluted sample/concentrate to 245 μL diluent, after this, the neat sample has been diluted at

100000 fold successfully.

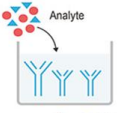
## General Operation Procedure

1. Determine wells for diluted standard, blank and sample. Add 100 μL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. It is recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Decant the liquid from each well, do not wash. Immediately add 100 μL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37 ℃.
3. Decant the solution from each well， add 350 μL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
4. Add 100 μL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37 ℃.
5. Decant the solution from each well, repeat the wash process for 5 times as conducted in step 3.
6. Add 90 μL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37 ℃. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.
7. Add 50 μL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

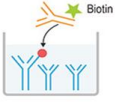
# Assay Procedure Summary



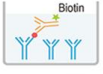
1.1 Take out the Micro ELISA pre-Plate, add 100μL Capture antibody working solution to each well. Incubate overnight at 2-8°C
1.2 Discard the liquid, add 200μL ELISA Plate Blocking Buffer to each well. Incubate for 60 min at 37°C



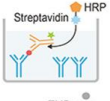

2. Discard the liquid, add 100μL standard or sample to the wells. Incubate for 90 min at 37°C



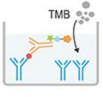

3. Discard the liquid, immediately add 100μL Biotinylated Detection Ab working solution to each well. Incubate for 60 min at 37°C



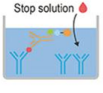

4. Aspirate and wash the plate for 3 times



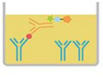

5. Add 100μL HRP conjugate working solution. Incubate for 30 min at 37°C. Aspirate and wash the plate for 5 times




6. Add 90μL Substrate Reagent. Incubate for 15 min at 37°C




7. Add 50μL Stop Solution



8. Read the plate at 450nm immediately. Calculation of the results

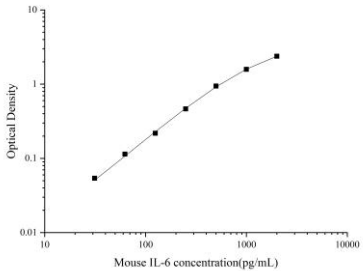
## Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four parameter logistic curve on log-log axis, with standard concentration on the x-axis and OD values on the y-axis.

If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

## Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

| pg/mL | OD | Corrected OD | Standard Curve |
|---|---|---|---|
| 2000 | 2.45 | 2.38 |  |
| 1000 | 1.655 | 1.585 | |
| 500 | 1.014 | 0.944 | |
| 250 | 0.534 | 0.464 | |
| 125 | 0.288 | 0.218 | |
| 62.5 | 0.184 | 0.114 | |
| 312.5 | 0.124 | 0.054 | |
| 0 | 0.07 | - | |



## Performance

- **Specificity**

This kit recognizes Mouse IL-6 in samples. No significant cross-reactivity or interference between Mouse IL-6 and analogues was observed.

## Troubleshooting

If the results are not good enough, please take pictures and save the experimental data in time. Keep the used plate and remaining reagents. Then contact our technical support to solve the problem. Meanwhile, you could also refer to the following materials.

| Problem | Causes | Solutions |
|---|---|---|
| Poor standard curve | The core reagent ratio is not appropriate | Adjust the capture antibody , detection antibody ratio and protein concentration |
| | Inaccurate pipetting | Check pipettes |
| | Improper standard dilution | Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing |
| | Wells are not completely aspirated | Completely aspirate wells in between steps |
| Low signal | Insufficient incubation time | Ensure sufficient incubation time |
| | Incorrect assay temperature | Use recommended incubation temperature. Bring substrate to room temperature before use |
| | Inadequate reagent volumes | Check pipettes and ensure correct preparation |
| | Improper dilution | |
| | HRP conjugate inactive or TMB failure | Mix HRP conjugate and TMB, rapid coloring |
| | The ratio of captured or detected antibodies is not appropriate | Adjust the capture or detection antibody ratio |
| Deep color but low value | Plate reader setting is not optimal | Verify the wavelength and filter setting on the Microplate reader |
| | | Open the Microplate Reader ahead to pre-heat |
| Large CV | Inaccurate pipetting | Check pipettes |
| | Experiment error or too long waiting time | Reduce experimental errors and waiting time |
| High background | The working concentration of antibodies or HRP conjugate is too high | Use the recommended dilution, or adjust the antibody or HRP conjugate ratio according to the results |
| | Plate is insufficiently washed | Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed |
| | Contaminated wash buffer | Prepare fresh wash buffer |
| | The buffer system is not suitable | Replace other buffer systems |
| Low sensitivity | Improper storage of the ELISA kit | All the reagents should be stored according to the instructions |
| | Stop solution is not added | Stop solution should be added to each well before measurement |

## 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. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
6. 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.
7. 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.
8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven't compared our products with those from other manufacturers.