Elabscience®

8th Edition, revised in October, 2018

（本试剂盒仅供体外研究使用，不用于临床诊断!）

小鼠白介素 2(IL-2)酶联免疫吸附测定试剂盒使用说明书

Mouse IL-2(Interleukin 2) ELISA Kit

产品货号：E-EL-M0042c

96T

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 027-65022280，027-87854967

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

QQ 客服 800110755

网址 www.elabscience.cn

联系时请提供产品货号(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、血浆或其他相关生物液体中IL-2浓度。

灵敏度、检测范围、特异性和重复性

●灵敏度：9.38pg/mL。

●检测范围：15.63-1000pg/mL。

●特异性：可检测样本中的小鼠 IL-2 且与其它相关蛋白无明显交叉反应。

●重复性：板内，板间变异系数均<10%。

背景介绍

白细胞介素-2 (IL-2)是一种白细胞介素，是免疫系统中的一种细胞因子信号分子，它是一种蛋白质，可以调节白细胞，通常是负责免疫的淋巴细胞等白细胞的活动。IL-2 是人体对微生物感染的自然反应的一部分，在区别异物(“非自我”)和“自我”时也是如此。IL-2 通过与淋巴细胞表达的 IL-2 受体结合来介导其作用。IL-2 对免疫系统产生广泛的影响，在调节免疫激活和稳态方面起着至关重要的作用。IL-2 及其多部分受体都是 I 型受体超家族的原型，这两种受体在临床上都得到了广泛的应用。

检测原理

本试剂盒采用双抗体夹心 ELISA 法。用抗 IL-2 抗体包被于酶标板上，实验时样品或标准品中的小鼠 IL-2 会与包被抗体结合，游离的成分被洗去。依次加入生物素化的抗小鼠 IL-2 抗体和辣根过氧化物酶标记的亲和素。抗 IL-2 抗体与结合在包被抗体上的 IL-2 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(TMB)，TMB 在辣根过氧化物酶的催化下呈现蓝色，加终止液后变成黄色。用酶标仪在 450nm 波长处测 OD 值，IL-2 浓度与 OD450 值之间呈正比，通过绘制标准曲线计算出样品中 IL-2 的浓度。

试剂盒组成及保存

未拆封的试剂盒可在 4℃保存一周；如果一周以后才使用试剂盒，请拆开试剂盒按照下表中的条件分别保存各组分。

中文名称	规格	保存条件
ELISA 酶标板 Micro ELISA Plate	8 孔×12 条	-20℃, 可存放 6 个月
冻干标准品 Reference Standard	2 支	
浓缩生物素化抗体(100×) Concentrated Biotinylated Detection Ab(100×)	1 支 120μL	
浓缩 HRP 酶结合物(100×) Concentrated HRP Conjugate(100×)	1 支 120μL	-20℃(避光), 可存放 6 个月
标准品&样品稀释液 Reference Standard & Sample Diluent	1 瓶 20mL	4℃,可存放 6 个月
生物素化抗体稀释液 Biotinylated Detection Ab Diluent	1 瓶 14mL	
酶结合物稀释液 HRP Conjugate Diluent	1 瓶 14mL	
浓缩洗涤液 Concentrated Wash Buffer	1 瓶 30mL	
底物溶液(TMB) Substrate Reagent	1 瓶 10mL	4℃(避光)
反应终止液 Stop Solution	1 瓶 10mL	4℃
封板覆膜 Plate Sealer	5 张	
产品说明书 Manual	1 份	
质检报告 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。

试验所需自备物品

1. 酶标仪(450nm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，双蒸水或去离子水
4. 吸水纸

注意事项

1.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。

2.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。

3.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。未用完的没有稀释的浓缩生物素化抗体(100×)、浓缩HRP酶结合物(100×)、酶标板及其他原液按照上述表格中保存条件存放。

4.检测使用的酶标仪需要安装能检测450±10nm波长的滤光片，光密度范围在0-3.5之间。

5.不同批号的试剂盒组份不能混用(洗涤液和反应终止液除外)。

6.试验中所用的EP管和吸头均为一次性使用，严禁混用。

样品收集方法

(具体处理方法可参考官网：http://www.elabscience.cn)

1.血清：全血样品于室温放置2小时或4℃过夜后于1000×g离心20分钟，取上清即可检测，收集血液的试管应为一次性的无内毒素试管。

2.血浆：抗凝剂推荐使用EDTA-Na_2，样品采集后30分钟内于1000×g离心15分钟，取上清即可检测。避免使用溶血，高血脂样品。

3.尿液：使用无菌容器收集尿液样本。在2-8℃下，1000×g离心15分钟以去除杂质，取上清即可检测。

4.唾液：收集样本后，在2-8℃下，4000×g离心10分钟以去除杂质，取上清即钟，取上清即可检测。避免使用溶血，高血脂样品。

样品注意事项

1.样品收集后若在1周内进行检测的可保存于4℃，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。

2.试剂盒检测范围不等同于样本的浓度范围，如果您的样品中检测物浓度高于标准品最高值，请根据实际情况，做适当倍数稀释(建议查阅文献后先做预实验，以确定稀释倍数)。

3.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。

4.若使用化学裂解液制备组织匀浆或细胞提取液，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。

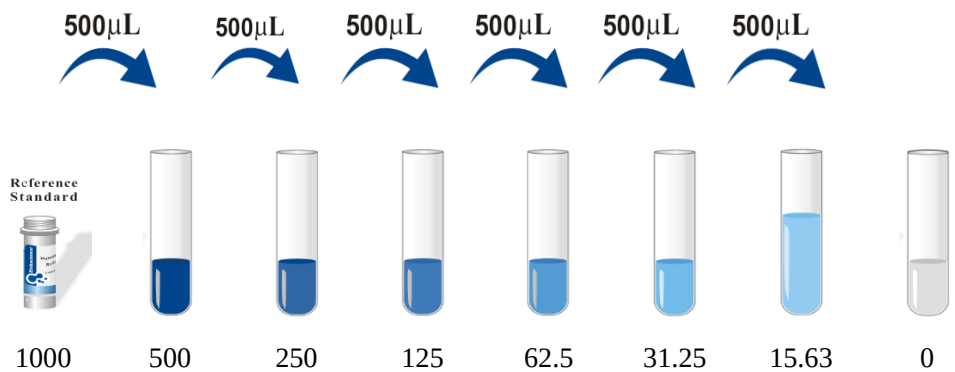
检测前准备工作：

1.提前20分钟从冰箱中取出试剂盒，平衡至室温。提前15分钟打开酶标仪预热。

2.**洗涤液：**将**浓缩洗涤液**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。

3.**标准品工作液：**将**标准品**于10000×g离心1分钟，加入**标准品&样品稀释液**1.0mL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，配成1000pg/mL的标准品工作液。然后根据需要进行倍比稀释。建议配制成以下浓度：1000、500、250、125、62.5、31.25、15.63、0 pg/mL。

倍比稀释方法：取7支EP管，每管中加入500μL**标准品&样品稀释液**，从1000pg/mL的标准品工作液中吸取500μL到其中一支EP管中混匀配成500pg/mL的标准品工作液，按此步骤往后依次吸取混匀。如下图。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。



4.**生物素化抗体工作液：**实验前计算当次实验所需用量(以100μL/孔计算)，实际配制时应多配制100-200μL。使用前15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。当日使用。

5.**酶结合物工作液：**实验前计算当次实验所需用量(以100μL/孔计算)，实际配制时应多配制100-200μL。使用前15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。当日使用。

操作步骤(操作一览表见第 10 页)

1.将**标准品工作液**依次加入到前两列孔中，每个浓度的工作液并列加两孔，每孔 100μL。待测样品加入到其他孔,每孔 100μL(**若样本浓度高于检测范围，需用标准品&样本稀释液稀释后取样**)。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.弃去液体，甩干，不用洗涤。每个孔中加入**生物素化抗体工作液** 100μL，混匀，酶标板加上覆膜，37℃温育 1 小时。

3.甩尽孔内液体，每孔加**洗涤液** 350μL，浸泡 1-2 分钟，吸去或甩掉酶标板内的液体，在厚的吸水纸上拍干。重复此洗板步骤 3 次。提示：此处与其他洗板步骤都可用洗板机。

4.每孔加**酶结合物工作液** 100μL，加上覆膜，37℃温育 30 分钟。

5.弃去孔内液体，甩干，洗板 5 次，方法同步骤 3。

6.每孔加**底物溶液(TMB)**90μL，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，即可终止。

7.每孔加**终止液** 50μL，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 450nm 波长测量各孔的光密度(OD 值)。

结果判断

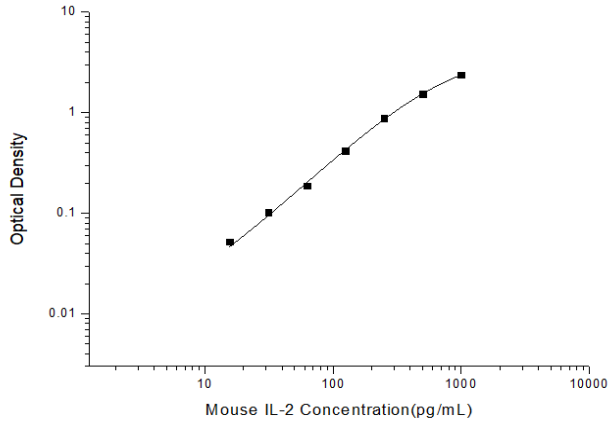
1.计算每组复孔的平均OD值。每个标准品的平均OD值减去空白孔的OD值作为矫正值。以浓度为横坐标，OD值为纵坐标，在双对数坐标纸上绘出四参数逻辑函数的标准曲线（作图时去掉空白组的值）。

2.若样品OD值高于标准曲线上限，应适当稀释后重测，计算浓度时应乘以稀释倍数。

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。

浓度：pg/mL	1000	500	250	125	62.5	31.25	15.63	0
OD	2.459	1.609	0.964	0.493	0.266	0.18	0.13	0.078
校正 OD	2.381	1.531	0.886	0.415	0.188	0.102	0.052	-



样本值

血清-本实验采用了来自小鼠的样本，以检测其中的小鼠 IL-2 水平。

样本类型	样本来源	样本值范围	稀释倍数
血清	脓毒症小鼠	71.8-231.2 pg/mL	1

检测健康小鼠血清 IL-2 的水平为 15.9 pg/mL，血浆样品低于检测限。

精密度

板内精密度:低浓度样本,中浓度样本和高浓度样本分别在1块板子上检测20次。

板间精密度:低浓度样本,中浓度样本和高浓度样本分别在3块板子上检测20次。

	批内变异系数			批间变异系数		
样本	1	2	3	1	2	3
数量	20	20	20	20	20	20
平均值pg/mL	49	87.4	385.6	52.4	82.2	365
标准差	3.3	3.8	20.4	3.1	4.8	15.7
变异系数 (%)	6.73	4.35	5.29	5.92	5.84	4.3

回收率

分别往 5 个不同样本中添加已知浓度的目标蛋白，做回收实验，得出回收率范围和平均回收率。

样本类型	回收率范围 (%)	平均回收率 (%)
血清(n=5)	87-101	93
血浆(EDTA)(n=5)	88-103	94
细胞上清(n=5)	89-102	96

线性

分别往5个样本中添加已知浓度的目标蛋白，做回收实验，得出回收率范围及平均回收率。将5个样本分别稀释2倍，4倍，8倍，16倍做回收实验，得出回收率范围及平均回收率。

		血清(n=5)	血浆(EDTA)(n=5)	细胞上清(n=5)
1:2	回收率范围(%)	88-101	93-107	93-107
	平均回收率(%)	95	100	99
1:4	回收率范围(%)	95-112	81-94	92-106
	平均回收率(%)	103	86	98
1:8	回收率范围(%)	97-111	84-97	95-110
	平均回收率(%)	105	89	101
1:16	回收率范围(%)	100-117	82-92	91-107
	平均回收率(%)	108	87	98

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	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
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
背景值高	检测抗体的工作浓度过高	使用推荐的稀释倍数
	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
灵敏度低	ELISA 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

操作概要

1. 在各孔中加入标准品或样品各 100μL，37℃孵育 90 分钟

2. 倒去孔内液体，加入 100μL 生物素化抗体工作液，37℃孵育 60 分钟

3. 洗涤 3 次

4. 加入 100μL 酶结合物工作液， 37℃孵育 30 分钟

5. 洗涤 5 次

6. 加入 90μL 底物溶液， 37℃孵育 15 分钟左右

7. 加入 50μL 终止液，立即在 450nm 波长处测量 OD 值

8. 结果计算

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.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密切相关，请务必准备充足的待测样品。

3.为了达到好的实验结果，请只使用 Elabscience 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。

4.由于操作过程中试剂制备以及酶标仪参数设置不正确，可能导致结果异常，实验前请仔细阅读说明书并调整好仪器。

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

Mouse IL-2(Interleukin 2) ELISA Kit

Catalog No: E-EL-M0042c

96T

Intended use

This ELISA kit applies to the in vitro quantitative determination of Mouse IL-2 concentrations in serum, plasma and other biological fluids.

Specification

- Sensitivity: 9.38pg/mL.
- Detection Range: 15.63-1000pg/mL
- Specificity: This kit recognizes Mouse IL-2 in samples. No significant cross-reactivity or interference between Mouse IL-2 and analogues was observed.
- Repeatability: Coefficient of variation is <10%.

Background

Interleukin-2 (IL-2) is an interleukin, a type of cytokine signaling molecule in the immune system. It is a protein that regulates the activities of white blood cells such as leukocytes, often lymphocytes that are responsible for immunity. IL-2 is part of the body's natural response to microbial infection, and in discriminating between foreign ("non-self") and "self". IL-2 mediates its effects by binding to IL-2 receptors, which are expressed by lymphocytes [1]. IL-2 exerts a wide spectrum of effects on the immune system, and it plays crucial roles in regulating both immune activation and homeostasis. Both IL-2 and its multipartite receptor are prototypical of the Type I receptor superfamily, and both have been exploited in numerous ways in the clinic [2].

1. Liao W , Lin J X , Leonard W J . IL-2 family cytokines: new insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation [J]. Current Opinion in Immunology, 2011, 23(5):0-604.
2. Gaffen S L, Liu K D. Overview of interleukin-2 function, production and clinical applications. [J]. Cytokine, 2004, 28(3):109-123.

Test principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Mouse IL-2. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Mouse IL-2 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Mouse IL-2, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ±2 nm. The OD value is proportional to the concentration of Mouse IL-2. You can calculate the concentration of Mouse IL-2 in the samples by comparing the OD of the samples to the standard curve.

Kit components & Storage

An unopened kit can be stored at 4℃ for 1 week. If the kit is not used within 1 week, store the items separately according to the following conditions once the kit is received.

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	8 wells ×12 strips	-20℃, 6 months
Reference Standard	2 vials	
Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 μL	
Concentrated HRP Conjugate (100×)	1 vial, 120 μL	-20℃(Protect from light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	4℃, 6 months
Biotinylated Detection Ab Diluent	1 vial, 14 mL	
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	4℃(Protect from light)
Stop Solution	1 vial, 10 mL	4℃
Plate Sealer	5 pieces	
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 supplies required

Microplate reader with 450 nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
Incubator capable of maintaining37℃
Deionized or distilled water
Absorbent paper
Loading slot for Wash Buffer

Note

1. 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.
2. A freshly opened ELISA Plate may appear to have a water-like substance, which is normal and will not have any impact on the experimental results.
3. Do not reuse the reconstituted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the above table.
4. The microplate reader should have a 450(±10 nm) filter installed and a detector that can detect the wavelength. The optical density should be within 0~3.5.
5. Do not mix or use components from other lots.
6. Change pipette tips in between adding standards, in between sample additions, and in between reagent additions. Also, use separate reservoirs for each reagent.

Sample collection

1. **Serum:** Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 20 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be non-endotoxin.
2. **Plasma:** Collect plasma using EDTA-Na2 as 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. Hemolysed samples are not suitable for ELISA assay!
3. **Saliva:** Remove particulates by centrifugation for 10 minutes at 4000×g at 2-8℃. Collect the supernatant to carry out the assay. Recommend to use fresh saliva samples.
4. **Urine:** Use a sterile container to collect urine samples. Remove particulates by centrifugation for 15 minutes at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

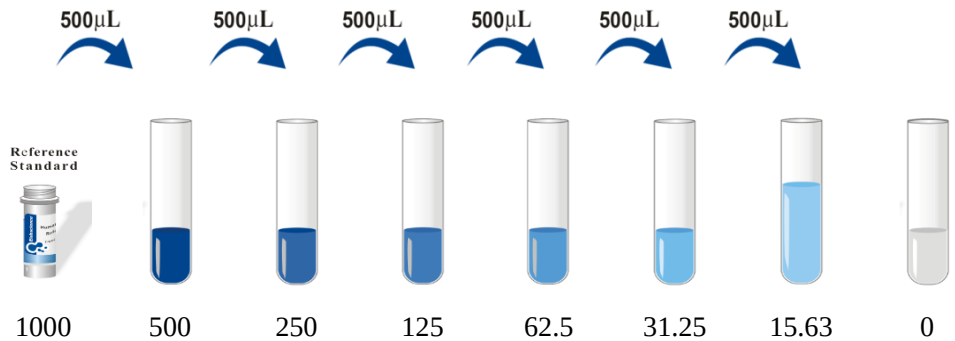
Note for sample

1. Samples should be assayed within 7 days when stored at 4℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles.
2. 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.
3. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
4. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, 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. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.
2. **Wash Buffer**: Dilute 30 mL of Concentrated Wash Buffer with720 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.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 1000pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 1000、500、250、125、62.5、31.25、15.63、0 pg/mL.

   Dilution method: Take 7 EP tubes, add 500μL of Reference Standard & Sample Diluent to each tube. Pipette 500μL of the 1000pg/mL working solution to the first tube and mix up to produce a 500pg/mL working solution. Pipette 500μL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.



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 stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.
5. **Concentrated HRP Conjugate working solution:** Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100×Concentrated HRP Conjugate to 1×working solution with Concentrated HRP Conjugate Diluent.

Assay procedure (A brief assay procedure is on the 19th page)

1. Add the **Standard working solution** to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100μL for each well). Add the samples to the other wells (100μL for each well). 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. Remove the liquid out of each well, do not wash. Immediately add 100 μL of **Biotinylated Detection Ab working solution** to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37 ℃.
3. Aspirate or decant the solution from each well， add 350 μL of **wash buffer** to each well. Soak for 1~2 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.
4. Add 100 μL of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37 ℃.
5. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 3.
6. Add 90 μL of **Substrate Reagent** to each well. Cover with a new plate 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 30min.
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.

Calculation of results

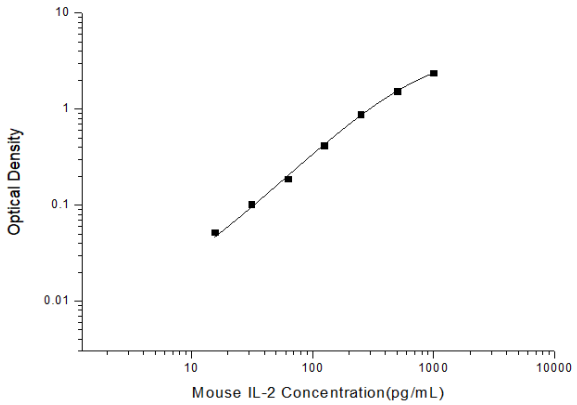
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. 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.

Concentration(pg/mL)	1000	500	250	125	62.5	31.25	15.63	0
OD	2.459	1.609	0.964	0.493	0.266	0.18	0.13	0.078
Corrected OD	2.381	1.531	0.886	0.415	0.188	0.102	0.052	-



Sample values

Serum–Samples from mice were evaluated for detectable levels of Mouse IL-2 in this assay.

Sample Type	Source	Range	Dilution Factor
Serum	Sepsis mouse	71.8-231.2 pg/mL	1

Serum from healthy mouse was measured 15.9 pg/mL while the plasma sample was below detection limit.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level Mouse IL-2 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level Mouse IL-2 were tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
mean(pg/mL)	49	87.4	385.6	52.4	82.2	365
Standard deviation	3.3	3.8	20.4	3.1	4.8	15.7
CV (%)	6.73	4.35	5.29	5.92	5.84	4.3

Recovery

The recovery of Mouse IL-2 spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=5)	87-101	93
EDTA plasma (n=5)	88-103	94
Cell culture media (n=5)	89-102	96

Linearity

Samples were spiked with high concentrations of Mouse IL-2 and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=5)	EDTA plasma (n=5)	Cell culture media (n=5)
1:2	Range (%)	88-101	93-107	93-107
	Average (%)	95	100	99
1:4	Range (%)	95-112	81-94	92-106
	Average (%)	103	86	98
1:8	Range (%)	97-111	84-97	95-110
	Average (%)	105	89	101
1:16	Range (%)	100-117	82-92	91-107
	Average (%)	108	87	98

Troubleshooting

Problem	Causes	Solutions
Poor standard curve	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.
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.
High background	Concentration of target protein is too high	Use recommended dilution factor.
	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.
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.

SUMMARY

1. Add 100μL standard or sample to each well. Incubate for 90min at 37℃.
2. Remove the liquid. Add 100μL Biotinylated Detection Ab. Incubate for 1 hour at 37℃.
3. Aspirate and wash 3 times.
4. Add 100μL HRP Conjugate. Incubate for 30 min at 37℃.
5. Aspirate and wash 5 times.
6. Add 90μL Substrate Reagent. Incubate for 15 min at 37℃.
7. Add 50μL Stop Solution. Read at 450nm immediately.
8. Calculation of results.

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. The final experimental results will be closely related to the validity of products, operational skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions!
4. 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.
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 equipments, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.