

(本试剂盒仅供体外研究使用, 不用于临床诊断!)

## **QuicKey-人中性粒细胞明胶酶相关脂质运载蛋白 (NGAL)酶联免疫吸附测定试剂盒使用说明书**

QuicKey Human NGAL (Neutrophil Gelatinase Associated Lipocalin) ELISA Kit

产品货号: E-TSEL-H0003

96T/48T/24T

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](mailto:Perry@elabscience.cn)

电子邮箱 (技术)                [techsupport@elabscience.cn](mailto:techsupport@elabscience.cn)

QQ 客服                        800110755

网址                              [www.elabscience.cn](http://www.elabscience.cn)

具体保质期请见试剂盒外包装标签。

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。

## QuicKey系列

与传统ELISA试剂盒相比，在实验时间节省至少1小时的同时，获得更加灵敏和精确的实验结果。Elabscience 自主开发的新技术，旨在帮助客户以更为高效的方式进行科学研究。

### 用途

该试剂盒用于体外定量检测人血清、血浆、尿液中NGAL 浓度。其它相关生物液体请咨询技术支持。

### 灵敏度、检测范围、特异性和重复性

- 灵敏度：0.06ng/mL。
- 检测范围：0.13-8ng/mL。
- 特异性：可检测样本中的人 NGAL ,且与其它类似物无明显交叉反应。
- 重复性：板内，板间变异系数均<10%。

### 背景介绍

中性粒细胞白明胶酶相关脂质运载蛋白 (NGAL), 或称 Lipocalin 2 和 Lcn2, 是一个大小约 25 kDa 的蛋白, 它是脂钙素超家族的一员, 是各种炎症过程的多效介质。NGAL 原是一种与人中性粒细胞基质金属蛋白酶 9 (MMP-9) 相关的蛋白。脂蛋白是一种胞外蛋白, 它们具有共同的三级结构, 形成类似于桶状的疏水配体结合位点。当与 MMP-9 结合时, NGAL 可保护其免受蛋白水解降解, 维持 MMP-9 的蛋白水解活性。NGAL 由多种类型的细胞释放, 是炎症、缺血、感染和肾脏损害的一种生物标志物。正如在肥胖和相关疾病中观察到的那样, 肠道和代谢炎症都与 NGAL 合成的增加有关。虽然肠道中的 NGAL 调节肠道菌群的组成并显示出抗炎活性, 但在其他实验环境中也表现出促炎活性。血浆 NGAL 是肾功能的一种新的标志物, 与等待肾移植的尿毒症患者终末期肾功能衰竭(ESRD)和血清肌酐的持续时间有关。移植患者血浆 NGAL 与同型半胱氨酸有关。

### 检测原理

本试剂盒采用双抗体夹心 ELISA 法。用抗人 NGAL 抗体包被于酶标板上, 实验时样品 (或标准品) 及生物素化的抗人 NGAL 抗体同时加入酶标板中, 样本 (或标准品) 中的人 NGAL 会与包被抗体结合, 同时抗人 NGAL 抗体与结合在包被抗体上的人 NGAL 结合, 游离的成分被洗去。再加入辣根过氧化物酶标记的亲合素, 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, NGAL 浓度与 OD<sub>450</sub> 值之间呈正比, 通过绘制标准曲线计算出样品中 NGAL 的浓度。

## 试剂盒组成及保存

未拆封的试剂盒可在 2-8℃保存 6 个月；试剂盒组分开封后请按照下表中的条件分别保存。

中文名称	规格	开封后保存条件
ELISA 酶标板 Micro ELISA Plate	96T: 8 孔×12 条 48T: 8 孔×6 条 24T: 8 孔×3 条	2-8℃, 可存放 1 个月
冻干标准品 Reference Standard	96T: 2 支 48T: 1 支 24T: 1 支	2-8℃, 复溶后当天使用
标准品&样品稀释液 Reference Standard & Sample Diluent	1 瓶 20mL	2-8℃
生物素化抗体工作液 Biotinylated Detection Ab Working Solution	1 瓶 6mL	
酶结合物稀释液 HRP Conjugate Diluent	1 瓶 14mL	
浓缩洗涤液 Concentrated Wash Buffer	1 瓶 30mL	
浓缩 HRP 酶结合物 (100×) Concentrated HRP Conjugate	96T: 1 支 120μL 48T: 1 支 60μL 24T: 1 支 60μL	2-8℃(避光)
底物溶液(TMB) Substrate Reagent	1 瓶 10mL	2-8℃
反应终止液 Stop Solution	1 瓶 10mL	
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: 0.5-10 $\mu$ L, 2-20 $\mu$ L, 20-200 $\mu$ L, 200-1000 $\mu$ L
3. 37 $^{\circ}$ C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和酶结合物。未用完的生物素化抗体工作液、浓缩HRP酶结合物(100 $\times$ )、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 $\pm$ 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(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/List-detail-241.html>)

1. 血清: 全血样品于室温放置2小时或2-8 $^{\circ}$ C过夜后于2-8 $^{\circ}$ C, 1000 $\times$ 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<sub>2</sub>, 样品采集后30分钟内于2-8 $^{\circ}$ C, 1000 $\times$ 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 $^{\circ}$ C下, 1000 $\times$ g离心15分钟以去除杂质, 取上清即可检测。

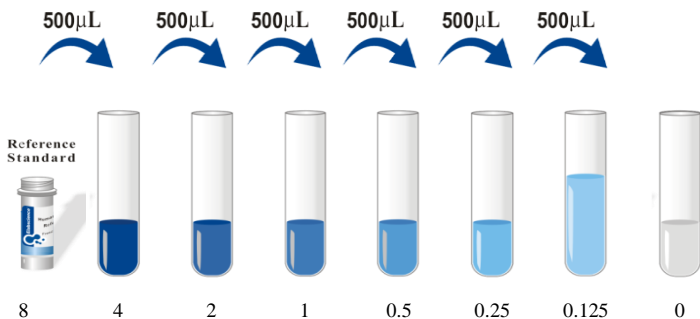
## 样品注意事项

1. 样品收集后若在1周内进行检测的可保存于2-8°C, 若不能及时检测, 请按一次使用量分装, 冻存于-20°C(1个月内检测), 或-80°C(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. 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°C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8ng/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: 8、4、2、1、0.5、0.25、0.125、0ng/mL。(操作图见下一页)

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8ng/mL的标准品工作液中吸取500μL到其中一支EP管中混匀配成4ng/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. **酶结合物工作液**：实验前计算当次实验所需用量(以100 $\mu$ L/孔计算)，实际配制时应多配制100-200 $\mu$ L。使用前15分钟，将**浓缩HRP酶结合物**于800 $\times$ g离心1分钟，以**酶结合物稀释液**将100 $\times$ **浓缩HRP酶结合物**稀释成1 $\times$ 工作浓度。当日使用。

### 操作步骤(第11页中附有简版操作概要)

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 50 $\mu$ L。待测样品加入到其他孔,每孔 50 $\mu$ L(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)。立即各孔中加入**生物素化抗体工作液** 50 $\mu$ L，给酶标板覆膜，37 $^{\circ}$ C 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，每孔加**洗涤液** 350 $\mu$ L，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。
3. 每孔加**酶结合物工作液** 100 $\mu$ L，加上覆膜，37 $^{\circ}$ C 温育 30 分钟。
4. 弃去孔内液体，甩干，洗板 5 次，方法同步骤 2。
5. 每孔加**底物溶液(TMB)**90 $\mu$ L，酶标板加上覆膜 37 $^{\circ}$ C 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。

6. 每孔加**终止液** 50 $\mu$ L，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。
7. 立即用酶标仪在 450nm 波长测量各孔的光密度(OD 值)。

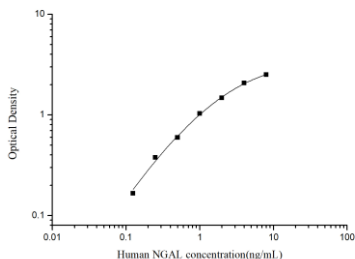
## 结果判断

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，应当适当稀释后重测。

## 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： ng/mL	8	4	2	1	0.5	0.25	0.125	0
OD	2.569	2.130	1.533	1.088	0.656	0.437	0.225	0.059
校正 OD	2.510	2.071	1.474	1.029	0.597	0.378	0.166	-



## 样本值

**血清/血浆/尿液-** 对健康志愿者的样本进行实验分析。

样本类型	范围	推荐稀释比例
血清 (n=19)	68.47-210.56ng/mL	50-500
血浆(EDTA) (n=12)	50-180.8ng/mL	50-500
尿液(n=17)	0.52-75ng/mL	1-50

## 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。

样本	批内变异系数			批间变异系数		
	1	2	3	1	2	3
数量	20	20	20	20	20	20
平均值(ng/mL)	0.37	0.90	3.57	0.35	0.81	3.73
标准差	0.02	0.04	0.16	0.02	0.04	0.17
变异系数 (%)	5.41	4.44	4.48	5.71	4.94	4.56



## 回收率

分别往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=8)	86-100	94
血浆(EDTA) (n=8)	101-110	104
尿液(n=8)	87-98	90

## 线性

分别往4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4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。

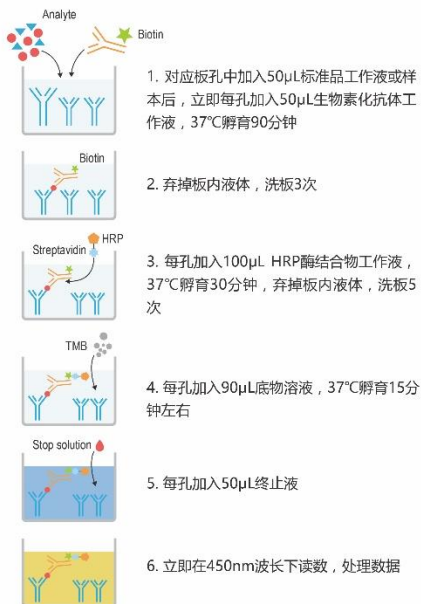
		血清 (n=4)	血浆(EDTA) (n=4)	尿液(n=4)
1:2	回收率范围(%)	88-99	90-101	101-110
	平均回收率(%)	91	96	104
1:4	回收率范围(%)	95-105	92-102	96-109
	平均回收率(%)	99	97	102
1:8	回收率范围(%)	87-96	86-100	98-111
	平均回收率(%)	92	93	105
1:16	回收率范围(%)	101-111	90-98	100-110
	平均回收率(%)	105	95	104

## 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



## 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。

## **QuicKey Human NGAL (Neutrophil Gelatinase Associated Lipocalin) ELISA Kit**

Catalog No: E-TSEL-H0003

96T/48T/24T

### **QuicKey Series**

Get more sensitive and precise results with saving at least 1h comparing to traditional ELISA Kits. The new developed technology in house will help to accelerate your science research in a more efficient way.

### **Intended use**

This ELISA kit applies to the in vitro quantitative determination of Human NGAL concentrations in serum, plasma, urine. Please consult technical support for the applicability if other biological fluids need to be tested.

### **Specification**

- Sensitivity: 0.06ng/mL.
- Detection Range: 0.13-8ng/mL
- Specificity: This kit recognizes Human NGAL in samples. No significant cross-reactivity or interference between Human NGAL and analogues was observed.
- Repeatability: Coefficient of variation is < 10%.

### **Background**

Neutrophil gelatinase associated lipocalin (NGAL) also known as Lipocalin 2 or Lcn2 is a 25 kDa, is a member of the lipocalin superfamily and a pleiotropic mediator of various inflammatory processes [1].NGAL, identified originally as a protein associated with matrix metalloproteinase 9 (MMP-9) of human neutrophils. Lipocalins are extracellular proteins which share a common tertiary structure that forms a barrel-like hydrophobic ligand binding site. When bound to MMP-9, NGAL protects it from proteolytic degradation sustaining the proteolytic activity of MMP-9. Neutrophil gelatinase-associated lipocalin (NGAL), is released by various cell types and is an attractive biomarker of inflammation, ischemia, infection, and kidney damage. Both intestinal and metabolic inflammation, as observed in obesity and related disorders, are associated with increased NGAL synthesis [2]. While NGAL in the intestinal tract regulates the composition of the gut microbiota and shows anti-inflammatory activities, it also exhibits

proinflammatory activities in other experimental settings. Plasma NGAL is a novel marker of kidney function, which correlates to duration of end-stage renal failure (ESRD) and serum creatinine in uremic patients awaiting kidney transplantation. Plasma NGAL is associated with homocysteine in transplanted patients.

1. Kjeldsen L, Cowland J B, Borregaard N. Human neutrophil gelatinase-associated lipocalin and homologous proteins in rat and mouse.[J]. *Biochim Biophys Acta*, 2000, 1482(1):272-283.
2. Tilg, H., & Moschen, A. R. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nature Reviews Immunology*, 2006, 6(10), 772.

### **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 Human NGAL. Samples (or Standards) and biotinylated detection antibody specific for Human NGAL are added to the micro ELISA plate wells. Human NGAL would combine with the specific antibody. Then 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 Human NGAL, 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  $450 \pm 2$  nm. The OD value is proportional to the concentration of Human NGAL. You can calculate the concentration of Human NGAL in the samples by comparing the OD of the samples to the standard curve.

## Kit components & Storage

An unopened kit can be stored at 2-8°C for six months. After test, the unused wells and reagents should be stored according to the table below.

Item	Specifications	Storage conditions after test
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips	2-8°C, 1 month
Reference Standard	96T: 2 vials 48T: 1 vial 24T: 1 vial	2-8°C, use the reconstituted standard within 24h
Reference Standard & Sample Diluent	1 vial, 20 mL	2-8°C
Biotinylated Detection Ab Working Solution	1 vial, 6 mL	
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 µL 48T: 1 vial, 60 µL 24T: 1 vial, 60 µL	2-8°C(Protect from light)
Substrate Reagent	1 vial, 10 mL	2-8°C
Stop Solution	1 vial, 10 mL	
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 450nm wavelength filter

High-precision transfer pipette, EP tubes and disposable pipette tips

Incubator capable of maintaining 37°C

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. Return the unused wells to the foil pouch provided in the kit, store it according to the conditions suggested in the above table.
3. Do not reuse the diluted standard and HRP conjugate working solution. The unspent biotinylated detection Ab working solution and other stock solutions should be stored according to the storage conditions suggested in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wave length at  $450 \pm 10$  nm. The optical density should be within 0-3.5.
5. Do not mix or use components with those from other lots.
6. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.

## Sample collection

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

**Serum:** Allow samples to clot for 2 hours at room temperature or overnight at  $2-8^{\circ}\text{C}$  before centrifugation for 20 min at  $1000 \times g$  at  $2-8^{\circ}\text{C}$ . Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be non-endotoxin.

**Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at  $1000 \times g$  at  $2-8^{\circ}\text{C}$  within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!

**Urine:** Use a sterile container to collect urine samples. Remove particulates by centrifugation for 15 minutes at  $1000 \times g$  at  $2-8^{\circ}\text{C}$ . Collect the supernatant to carry out the assay.

## Note for sample

1. Samples should be assayed within 7 days when stored at 2-8°C, otherwise samples must be divided up and stored at -20°C ( $\leq 1$  month) or -80°C ( $\leq 3$  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. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

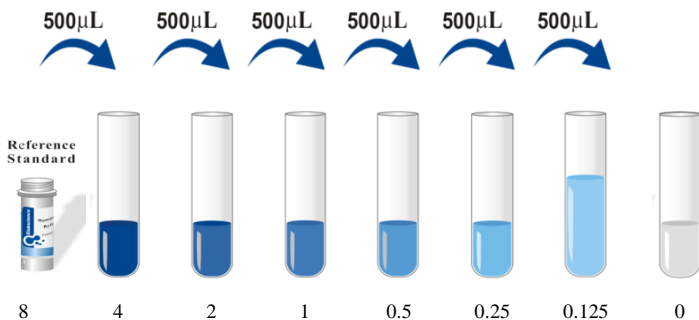
## Reagent preparation

1. Bring all reagents to room temperature (18-25°C) 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 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°C water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000 $\times$ g for 1 min. Add 1.0 mL of Reference Standard & 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 8ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 8、4、2、1、0.5、0.25、0.125、0ng/mL.

Dilution method: Take 7 EP tubes, add 500 $\mu$ L of Reference Standard & Sample Diluent to each tube. Pipette 500 $\mu$ L of the 8ng/mL working solution to the first tube and mix up to produce a 4ng/mL working solution. Pipette 500 $\mu$ L of the solution from the former tube into the latter one according to this step. 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. (The operation diagram is shown on the next page)

4. **HRP Conjugate working solution:** Calculate the required amount before the experiment (100  $\mu$ L/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800 $\times$ g for 1 min, then dilute the 100 $\times$  Concentrated HRP Conjugate to 1 $\times$  working solution with HRP Conjugate Diluent.





**Assay procedure** (A brief assay procedure is on the 22<sup>th</sup> 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 (50 µL for each well). Add the samples to the other wells (50 µL for each well). Immediately add 50µL of **Biotinylated Detection Ab working solution** to each well. Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37°C. 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. 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.
3. Add 100µL of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37 °C.
4. Aspirate or decant the solution from each well, repeat the wash process for 5 times as conducted in step 2.
5. Add 90µL of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37 °C. 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.
6. Add 50µL of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

## Calculation of results

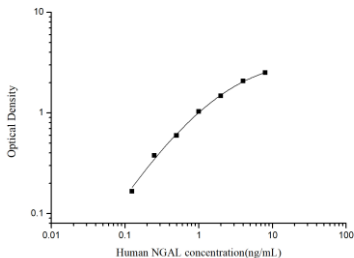
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

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(ng/mL)	8	4	2	1	0.5	0.25	0.125	0
OD	2.569	2.130	1.533	1.088	0.656	0.437	0.225	0.059
Corrected OD	2.510	2.071	1.474	1.029	0.597	0.378	0.166	-



## Sample values

**Serum/Plasma/Urine**–Samples from apparently healthy volunteers were evaluated for the presence of Human NGAL in this assay.

Sample Type	Source	Range	Dilution Factor
Serum(n=19)	Healthy human	68.47-210.56ng/mL	50-500
EDTA plasma (n=12)	Healthy human	50-180.8ng/mL	50-500
Urine(n=17)	Healthy human	0.52-75ng/mL	1-50

## Precision

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

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

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(ng/mL)	0.37	0.90	3.57	0.35	0.81	3.73
Standard deviation	0.02	0.04	0.16	0.02	0.04	0.17
CV (%)	5.41	4.44	4.48	5.71	4.94	4.56

## Recovery

The recovery of Human NGAL 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=8)	86-100	94
EDTA plasma (n=8)	101-110	104
Urine(n=8)	87-98	90

## Linearity

Samples were spiked with high concentrations of Human NGAL and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

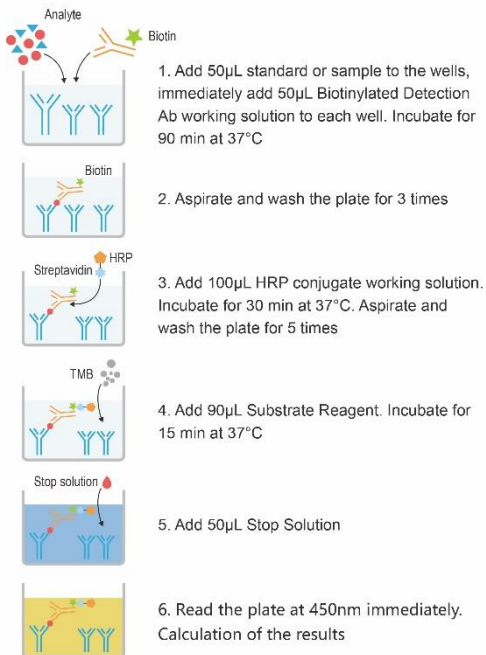
		Serum (n=4)	EDTA plasma (n=4)	Urine(n=4)
1:2	Range (%)	88-99	90-101	101-110
	Average (%)	91	96	104
1:4	Range (%)	95-105	92-102	96-109
	Average (%)	99	97	102
1:8	Range (%)	87-96	86-100	98-111
	Average (%)	92	93	105
1:16	Range (%)	101-111	90-98	100-110
	Average (%)	105	95	104

## 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	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



## 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.