



# Human KGF ELISA Kit

For the quantitative in vitro determination of Human Keratinocyte Growth Factor  
concentrations in

**serum - plasma - tissue homogenates - other biological fluids**

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

*This package insert must be read in its entirety before using this product.*

**ELISA**

**ENZYME LINKED IMMUNOSORBENT ASSAY**



## INTENDED USE AND TEST PRINCIPLE

This KGF 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 KGF in the sample, this KGF 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 KGF concentration. The concentration of KGF in the samples is then determined by comparing the O.D. of the samples to the standard curve.

## SAMPLE COLLECTION AND STORAGEES

**Serum** - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

**Tissue homogenates** - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

**Cell culture supernates and other biological fluids** - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or



-80°C for later use. Avoid repeated freeze/thaw cycles.



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

## **SAMPLE PREPARATION**

1. We are only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. Sample should be diluted by 0.01mol/L PBS(PH=7.0-7.2).
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals.
5. Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
6. Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernatant may not be detected by the kit.
7. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

## **MATERIALS REQUIRED BUT NOT SUPPLIED**

1. 37 °C incubator
2. Standard microplate reader capable of measuring absorbance at 450 nm
3. Precision pipettes, disposable pipette tips and Absorbent paper
4. Distilled or deionized water



## REAGENTS PROVIDED

*All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.*

Name	96 determinations	48 determinations
MICROTITER PLATE	12*8strips	12*4strips
STANDARD (6 vial)	0.3ml/vial	0.3ml/vial
SAMPLE DILUENT	6.0ml	3.0ml
ENZYME CONJUGATE	10.0ml	5.0ml
WASH SOLUTION	25ml	15ml
SUBSTRATE A	6.0ml	3.0ml
SUBSTRATE B	6.0ml	3.0ml
STOP SOLUTION	6.0ml	3.0ml
Closure plate membrane	2	2
User manual	1	1
Sealed bags	1	1

### Note:

1. Standard concentration was followed by: 800, 400, 200, 100, 50, 25 pg/mL.
2. If samples generate values higher than the highest standard, please dilute the samples with Sample Diluent and repeat the assay.

## PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting

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**Address:** Kenneth G. Howell , 748 Nickel Road , San Francisco(415) , CA 94104

**Telephone:** 0014159141567



disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from Rat blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

9. All samples should be disposed of in a manner that will inactivate viruses.
10. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
11. Substrate Solution is easily contaminated. If bluish prior to use, do not use.
12. Substrate B contain 20% acetone, keep this reagent away from sources of heat or flame.
13. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C).

## **REAGENT PREPARATION AND STORAGE**

**Wash Solution (1X)** - Dilute 1 volume of Wash solution (20X) with 19 volumes of deionized or distilled water. Wash Solution is stable for 1 month at 2-8°C.

## **ASSAY PROCEDURE**

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microtiter plate.
2. Add 50µl of Standard or Sample to the appropriate wells. Blank well doesn't add anything.
3. Add 100µl of Enzymeconjugate to standard wells and sample wells except the blank well, cover with an adhesive strip and incubate for 60 minutes at 37°C.
4. Wash the Microtiter Plate 4 times.

**Manual Washing** - Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Solution (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for a total of four times. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when



washing the plate to assure that all strips remain securely in frame.

**Automated Washing** - Aspirate all wells, then wash plates four times using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 $\mu$ L/well/wash. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

5. Add Substrate A 50 $\mu$ l and Substrate B 50 $\mu$ l to each well. Gently mix and incubate for 15 minutes at 37°C. **Protect from light.**
6. Add 50 $\mu$ 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.
7. 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 (X) axis versus the corresponding concentration on the horizontal (Y) 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 blank well 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. Intra-assay CV(%) is less than 10% and Inter-assay CV(%) is less than 15%.
6. Assay range: 25 pg/mL – 800 pg/mL.
7. Sensitivity: The minimum detectable dose of Human KGF is typically less than 1.0 pg/mL.



8. Cross-reactivity: This assay recognizes recombinant and natural Human KGF. No significant cross-reactivity or interference was observed.
9. Storage: 2-8°C (Use frequently); six months (-20°C)。
10. Standard curve



## IMPORTANT NOTE

1. Limited by the current condition and scientific technology, we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.
2. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
3. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to the instruction attached in kit while electronic ones from our website is only for information.
4. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.



## TROUBLE SHOOTING

*If the results are not good, please take pictures, keep the used plate and remaining reagents. Then contact our technical department. Meanwhile, you could refer to the following materials.*

<b>Problem</b>	<b>Possible Source</b>	<b>Correction Action</b>
Poor Standard Curve	Incomplete washing and aspiration	Adequate washing and adequate aspiration
	Inaccurate Pipetting	Check and Calibrate pipettes
Poor Precision	Incomplete washing of wells	Ensure sufficient washing
	Inadequate mixing and aspiration reagents	Adequate aspiration and mixing reagents
	Reused pipette tips, containers and sealers	Change and use new pipette tips, containers and sealers
	Inaccurate Pipetting	Check and Calibrate pipettes
Low O.D Values	Inadequate reagent volumes added to wells	Calibrate pipettes and Add adequate reagents
	Incorrect incubation times	Ensure sufficient incubation times
	Incorrect incubation temperature	Reagents balanced to room temperature
	Conjugate or substrate reagent failure	Mix conjugate & substrate, color should develop immediately
	No stop solution added	Follow the assay protocol in the kit manual
	Read beyond suggested reading time	Read within the time recommended in the manual
Sample Values	Improper Sample Storage	Store the sample properly and use the fresh sample
	Improper sample collection and preparation	Take proper sample collection and preparation method
	Low quantity of analyte in samples	Use new sample and repeat assay

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





# 人角化细胞生长因子（KGF）试剂盒（ELISA）

## 使用说明书

- 本试剂盒用于体外定量检测血清、血浆、组织匀浆及相关液体样本中人角化细胞生长因子（KGF）的含量。
- 有效期：6个月
- 保存条件：2-8℃
- 本试剂盒仅供体外研究使用，不用于临床诊断

### 实验原理

试剂盒采用双抗体一步夹心法酶联免疫吸附试验（ELISA）。往预先包被人角化细胞生长因子（KGF）捕获抗体的包被微孔中，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人角化细胞生长因子（KGF）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。

### 样本处理及要求

1. **血清**：将收集于血清分离管的全血标本在室温放置2小时或4℃过夜，然后1000×g离心20分钟，取上清即可，或将上清置于-20℃或-80℃保存，但应避免反复冻融。
2. **血浆**：用EDTA或肝素作为抗凝剂采集标本，并将标本在采集后的30分钟内于2-8℃ 1000×g离心15分钟，取上清即可检测，或将上清置于-20℃或-80℃保存，但应避免反复冻融。
3. **组织匀浆**：用预冷的PBS (0.01M, pH=7.4)冲洗组织，去除残留血液（匀浆中裂解的红细胞会影响测量结果），称重后将组织剪碎。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，于冰上充分研磨。为了进一步裂解组织细胞，可以对匀浆液进行超声破碎，或反复冻融。最后将匀浆液于5000×g离心5~10分钟，取上清检测。
4. **细胞培养物上清或其它生物标本**：请1000×g离心20分钟，取上清即可检测，或将上清置于-20℃或-80℃保存，但应避免反复冻融。

注：标本溶血会影响最后检测结果，因此溶血标本不宜进行此项检测。



### 标本处理

1. 本公司只对试剂盒本身负责，不对因使用该试剂盒所造成的样本消耗负责，请使用者使用前充分考虑到样本的可能使用量，预留充足的样本。
2. 实验前应预测标本含量，如果标本浓度过高，应对标本进行稀释，使稀释后的标本符合试剂盒的检测范围，计算时再乘以相应的稀释倍数。标本使用0.01mol/L的PBS稀释(PH=7.0-7.2)。
3. 若所检样本不包含在说明书所列样本之中，建议进行预实验验证其有效性，并注意留存样本。
4. 使用化学裂解液制备的组织匀浆或细胞提取液可能会由于某些化学物质的引入导致ELISA实验结果偏差。
5. 若样本为细胞培养上清，因该类样本干扰因素较多，如：细胞状态、细胞数量、采样时间等，所以可能存在检测不出的情况。
6. 某些天然蛋白或重组蛋白，包括原核及真核重组蛋白，可能因为与本产品所使用的检测抗体及捕获抗体不匹配，而不被检测出。
7. 建议使用新鲜样本，保存时间过长可能会存在蛋白降解或变性导致实验结果偏差。

### 需要而未提供的试剂和器材

1. 酶标仪（450nm）
2. 高精度加样器及枪头：0.5-10uL、2-20uL、20-200uL、200-1000uL
3. 37℃恒温箱
4. 蒸馏水或去离子水

### 试剂盒组成

名称	96孔配置	48孔配置	备注
微孔酶标板	12孔×8条	12孔×4条	无
标准品	0.3mL*6管	0.3mL*6管	无
样本稀释液	6mL	3mL	无
检测抗体-HRP	10mL	5mL	无
20×洗涤缓冲液	25mL	15mL	按说明书进行稀释
底物A	6mL	3mL	无
底物B	6mL	3mL	无
终止液	6mL	3mL	无
封板膜	2张	2张	无
说明书	1份	1份	无
自封袋	1个	1个	无

### 备注：

1. 标准品浓度依次为：800、400、200、100、50、25 pg/mL
2. 经过大量正常标本检验，标本的正常浓度值均在试剂盒提供的检测范围内，实验过程中直接取50μL样本上样即可。当有部分样本值超过最大标准品浓度时，可用样本稀释液将标本进行适当稀释后再进行实验。



### 注意事项

1. 严格按照规定的时间和温度进行温育以保证准确结果。所有试剂都必须在使用前达到室温 20-25℃。使用后立即冷藏保存试剂。
2. 洗板不正确可以导致不准确的结果。在加入底物前确保尽量吸干孔内液体。温育过程中不要让微孔干燥掉。
3. 消除板底残留的液体和手指印，否则影响 OD 值。
4. 底物显色液应呈无色或很浅的颜色，已经变蓝的底物液不能使用。
5. 避免试剂和标本的交叉污染以免造成错误结果。
6. 在储存和温育时避免强光直接照射。
7. 平衡至室温后再打开密封袋以防水滴凝聚在冷板条上。
8. 任何反应试剂不能接触漂白溶剂或漂白溶剂所散发的强烈气体。任何漂白成分都会破坏试剂盒中反应试剂的生物活性。
9. 不能使用过期产品。
10. 如果可能传播疾病，所有的样品都应管理好，按照规定的程序处理样品和检测装置。

### 试剂准备

试剂盒从冷藏环境中取出应在室温平衡后方可使用。

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

### 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℃。
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 50μL；
3. 样本孔中加入待测样本 50μL；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 100μL，用封板膜封住反应孔，37℃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（350μL），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 A、B 各 50μL，37℃避光孵育 15min。
7. 每孔加入终止液 50μL，15min 内，在 450nm 波长处测定各孔的 OD 值。

### 实验结果计算

以所测标准品的OD值为横坐标，标准品的浓度值为纵坐标，在坐标纸上或用相关软件绘制标准曲线，并得到直线回归方程，将样品的OD值代入方程，计算出样品的浓度。

### 试剂盒性能

1. 检测范围：25 pg/mL – 800 pg/mL。
2. 灵敏度：最低检测浓度小于 1.0 pg/mL。
3. 特异性：不与其它可溶性结构类似物交叉反应。
4. 重复性：板内变异系数小于 10% ，板间变异系数小于 15% 。



## 说明

1. 由于现有条件及科学技术水平尚不能对所有供货商提供的所有原料进行全面的鉴定与分析，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，请务必准备充足的标本备份。
3. 不同批次的同一产品可能会有少许差别，如：检测限、灵敏度以及显色时间等，请依据试剂盒内说明书进行实验操作，网站电子版说明书仅作参考。
4. 只有全部使用本试剂盒配套试剂才能保证检测效果，不能混用其他制造商的产品。只有严格遵守本试剂盒的实验说明才会得到最佳的检测结果。

## 问题解答

若实验效果不好，请及时对显色结果拍照，保留所用板条及未使用试剂，并妥善保存，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	可能原因	解决方案
标准曲线差	吸取及洗涤不充分	充分的吸取及洗涤
	移液不精确	检查和校正移液器
精密度低	洗涤不充分	按说明书要求充分洗涤和浸泡
	混匀不充分和吸取试剂不足	充分混匀和吸取试剂
	重复利用吸头、容器和覆膜	使用加样器要更换新的吸头、使用新的容器和覆膜
O.D 值低	加样不精确	检查和校正移液器
	每孔加的试剂量不精确	校正移液器，精确加入试剂
	温育时间不正确	保证充足的温育时间
	温育温度不正确	试剂要平衡至室温并保证准确的温育温度
	酶标记物或底物失效	通过混合酶标记物和底物，颜色应迅速显现来检查
样本值	没有加入终止液	按照说明书实验操作步骤加入终止液
	超出读数时间读数	在说明书推荐的读数时间内读数
	不正确的样本储存方式	正确储存样本，使用新鲜样本进行实验
样本值	不正确的样本收集和处理方法	采取正确的样本收集和处理方法
	待测物质在样本中含量低	使用新鲜样本，重复实验