



低氧诱导蛋白 1- α 定量分析酶联免疫 检测试剂盒

本试剂盒采用识别人和小鼠HIF-1 α 蛋白中同时都具有的位点的抗体，能够人和小鼠两个种属通用。本试剂盒仅供科研使用。用于体外定量检测人、小鼠血清、血浆或细胞培养上清液中的HIF-1 α 浓度。**使用前请仔细阅读说明书并检查试剂组分是否完整。**如有产品包装破损或质量投诉，请在收到货一个月之内联系我们。

HIF-1 α 简介：

低氧诱导蛋白 1- α (HIF-1 α)，是一个有 735 个氨基酸的胞质内和胞核内的蛋白。它由肾脏或心脏的许多组织高水平表达。在肿瘤的发生和转移过程中会过表达，肿瘤内的低氧环境会刺激其产生，并作为刺激原可相应刺激肿瘤蛋白及肿瘤抑制蛋白的编码及表达。

HIF-1 α 的功能是作为一个重要的转录调控因子以应对低氧环境。HIF-1 α 可相应地活化与转录有关的四十种基因，包括红细胞生成素、糖转运相关、糖分解相关的酶、血管内皮细胞生长因子和与氧转运和代谢相关的蛋白。HIF-1 α 在胚胎的血管生成、肿瘤血管生成和肿瘤的发展以及其它缺血性病症中均有重要的作用。

检测原理：

本试剂盒采用双抗体夹心ELISA法检测样本中HIF-1 α 的浓度。HIF-1 α 捕获抗体已预包被于酶标板上，当加入标本或参考品时，其中的HIF-1 α 会与捕获抗体结合，其它游离的成分通过洗涤的过程被除去。当加入生物素化的抗HIF-1 α 抗体后，抗HIF-1 α 抗体与HIF-1 α 结合，形成夹心的免疫复合物，其它游离的成分通过洗涤的过程被除去。随后加入辣根过氧化物酶标记的亲合素。生物素与亲合素特异性结合，亲合素连接的酶就会与夹心的免疫复合物连接起来；其它游离的成分通过洗涤的过程被除去。最后加入显色剂，若样本中存在HIF-1 α 将会形成免疫复合物，辣根过氧化物酶会催化无色的显色剂氧化成蓝色物质，在加入终止液后呈黄色。通过酶标仪检测，读其450nm处的OD值，HIF-1 α 浓度与OD₄₅₀值之间呈正比，通过参考品绘制标准曲线，对照未知样本中OD值，即可算出标本中HIF-1 α 浓度。

HIF-1 α 定量分析酶联免疫检测试剂盒组成：

| 组分 | 规格 (96T/48T) |
|-----------------------------------|-------------------------|
| HIF-1 α 预包被板 | 12 条/6 条 |
| 标准品稀释液 | 10ml/5ml |
| 样本分析缓冲液 | 5ml/3ml |
| HIF-1 α 标准品 | 2 支/1 支(冻干粉) |
| 50 \times HIF-1 α 生物素化抗体 | 210 μ l/105 μ l |
| 生物素化抗体稀释液 | 10ml/5ml |
| HRP 连接的酶结合物 | 10ml/5ml |
| 浓缩洗涤液 20 \times | 30ml/15ml |
| TMB 底物 | 10ml/5ml |
| 中止液 | 5ml/3ml |
| 封板胶纸 | 3/2 张 |
| 说明书 | 1 份 |

标本收集：

- 标本的收集请按下列流程进行操作：
 - 细胞上清标本离心去除悬浮物后即可；
 - 血清标本应是自然凝固后，取上清，避免在冰箱中凝固血液；
 - 血浆标本，推荐用EDTA的方法收集
 - 若待测样本不能及时检测，标本收集后请分装，冻存于-20 $^{\circ}$ C，避免反复冻融。
- 血清标本不应添加任何防腐剂或抗凝剂；
- 标本应清澈透明，检测前样本中如有悬浮物应通过离心去除。
- 请勿使用溶血，高血脂或污染的标本检测，否则结果将不准确。



5. HIF-1 α 作为胞内蛋白，一般细胞裂解或组织匀浆后测定。

推荐细胞裂解时加如下酶抑制剂或其它试剂以保护蛋白不被相应的酶降解：10%(w/v) glycerol, 3mM EDTA, 1 mM MgCl₂, 20mM β -glycerophosphate, 25mM NaF, 1% TritonX-100, 25 μ g/ml Leupeptin, 25 μ g/ml Pepstatin, and 3 μ g/ml Aprotinin

注：血清或血浆样本、组织匀浆或细胞匀浆样本请用样本分析缓冲液做稀释后再检测

注意事项：

1. 试剂盒请保存在2~8℃。
2. 浓缩洗涤液因在低温下可能有结晶，请水浴加热使结晶完全溶解后再配制工作液。
3. 标准品复溶加样后，剩余部份请丢弃。
4. 底物请勿接触氧化剂和金属。
5. 加样时，请及时更换枪头，避免交叉污染。
6. 严禁混用不同批号的试剂盒组份。
7. 充分混匀对保证反应结果的准确性很重要，在加液后请轻轻叩击边缘以保证混匀。
8. 室温反应，请严格控制在25~28℃。
9. 洗涤过程是至关重要的，洗涤不充分会使精确度下降并导致结果误差较大。
10. 试验中标准品和样本检测时建议作双复孔。
11. 加样过程中避免气泡的产生。
12. 血清和血浆标本的检测时，检测抗体的孵育时间应适当延长。

检测前准备工作：

1. 试剂盒自冰箱中取出后应置室温（25-28℃）平衡20分钟；每次检测后剩余试剂请及时于2~8℃保存。
2. 将浓缩洗涤液用双蒸水或去离子水稀释（1份加19份水）。
3. 如有5X准品稀释液，请按所需量用双蒸水或去离子水稀释（1份加4水）。
4. 标准品：按标签复溶体积加入标准品稀释液复溶使HIF-1 α 终浓度达到8000pg/ml，室温反应，请严格控制在25~28℃，静置15~20分钟后轻轻混悬（建议抽吸几次）待彻底溶解，用标准品稀释液倍比梯度稀释后依次加入检测孔中。（标准曲线取七个点，最高浓度为8000 pg/ml，标准品稀释液直接加入作为0浓度。）



5. 检测抗体，加样前，按1：50配制所需的工作液。

洗涤方法：

自动洗板机或人工洗板：每孔洗涤液为300 μ l，注入与吸出间隔15-30秒。洗板5次。最后一次洗板完成后将板倒扣着在厚吸水纸上用力拍干。

实验过程需自备的材料：

1. 不同规格的加样枪及相应的枪头；
2. 酶标仪；
3. 自动洗板机；
4. 去离子水或双蒸水；

操作步骤：

1. 通过计算并确定一次性实验所需的板条数，取出所需板条放置在框架内，暂时用不到板条请放回铝箔袋密封，保存于4℃。
2. 建议设置本底校正孔，即空白孔，设置方法为该孔只加TMB显色液和终止液。每次实验均需做标准品对照并画出标准曲线。
3. 分别将标本或不同浓度标准品（100 μ l/孔）加入相应孔中，血清血浆标本请用样本分析缓冲液从20倍开始稀释后加样。室温（25-28℃）孵育120分钟。
4. 洗板3次，且最后一次置厚吸水纸上拍干。
5. 加入稀释好的生物素化抗体工作液（100 μ l/孔）。用封板胶纸封住，室温（25-28℃）孵育60分钟。



6. 洗板3次，且最后一次置厚吸水纸上拍干。
7. 加入亲和素连接的HRP酶工作液(100u1/孔)。用封板胶纸封住反应孔，避光室温（25-28℃）孵育20分钟。
8. 洗板3次，且最后一次置厚吸水纸上拍干。
9. 加入显色剂TMB100u1/孔，避光室温（25-28℃）孵育10分钟。
10. 加入中止液50u1/孔，混匀后即刻测量OD₄₅₀值。

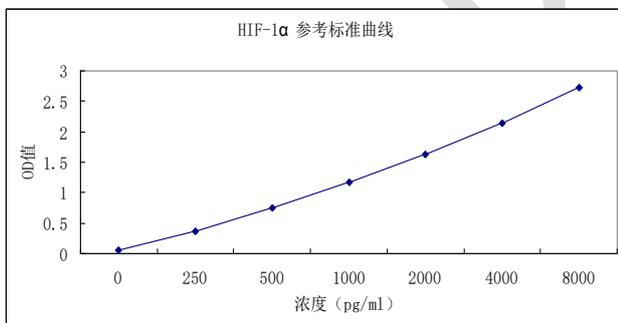
结果判断：

1. 复孔的值在20%的差异范围内结果才有效，复孔的值平均后可作为测量值。
2. 每个标准品或标本的OD值应减去本底校正孔的OD值。
3. 手工绘制标准曲线。以标准品浓度作横坐标，OD值作纵坐标，以平滑线连接各标准品的坐标点。通过标本的OD值可在标准曲线上查出其浓度。
4. 若标本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。

典型数值和参考曲线

| 浓度pg/ml | 典型OD值1 | 典型OD值2 | OD平均值 |
|---------|--------|--------|--------|
| 0 | 0.0578 | 0.0524 | 0.0551 |
| 250 | 0.3712 | 0.3618 | 0.3665 |
| 500 | 0.7846 | 0.7264 | 0.7555 |
| 1000 | 1.1924 | 1.1496 | 1.171 |
| 2000 | 1.6382 | 1.6216 | 1.6299 |
| 4000 | 2.1563 | 2.1119 | 2.1341 |
| 8000 | 2.7537 | 2.6955 | 2.7246 |

HIF-1 α 参考标准曲线



注意：本图仅供参考，应以同次试验标准品所绘标准曲线计算标本含量。

灵敏度，特异性和重复性：

1. 灵敏度：多次重复结果表明，最小检出量为43pg/ml.
2. 特异性：与HIF-2 α 不反应。识别位点人与小鼠通用。
3. 重复性：板内，板间变异系数均<10%.

参考文献：

1. Carbia-Nagashima A. et al., 2007,Cell 131:309-23.
2. Cho S. et al., 2001, FEBS Lett. 498:62-6.
3. Masson N. et al., 2001,EMBO J. 20:5197-206.
4. Bae S.-H. et al., 2004, Biochem. Biophys. Res. Commun. 324:394-400



ELISA Kit for the Quantitative Analysis of HIF-1 α

The HIF-1 α ELISA (enzyme-linked immunosorbent assay) kit is used for detection of HIF-1 α in cell culture supernatants, serum and plasma. **THE ELISA KIT IS FOR RESEARCH USE ONLY.** Please read this instruction manual carefully and check out the material provided before use, and you can contact with our company if any questions. You can enter our website or call us for other aim.

Introduction

Hypoxia-inducible factor 1-alpha, also known as HIF-1-alpha, is a 735aa cytoplasm and nucleus protein. It is expressed in most tissues with highest levels in kidney and heart. It is overexpressed in the majority of common human cancers and their metastases, due to the presence of intratumoral hypoxia and as a result of mutations in genes encoding oncoproteins and tumor suppressors.

HIF1 α functions as a master transcriptional regulator of the adaptive response to hypoxia. HIF1 α activates the transcription of over 40 genes, including, erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia. HIF1 α plays an essential role in embryonic vascularization, tumor angiogenesis and pathophysiology of ischemic disease.

Principles of the Test

The kits is a solid sandwich enzyme-linked immunosorbent assay for detection of HIF-1 α . An anti-HIF-1 α monoclonal antibody has been absorbed onto the wells of the microtiter strips provided. Samples including specimens or standards were pipetted into wells. The HIF-1 α in specimens or standards would be captured by the coated antibody and the free others were removed by washing. The HIF-1 α biotin-conjugated antibody were added and binds to HIF-1 α captured by the first antibody, which formed a sandwich. Streptavidin-HRP would be added and binds to the biotin conjugated antibody, then free Streptavidin-HRP would be removed during a wash step. After this, substrate solution would be added and catalyzed by the HRP, and a coloured product is formed. The intensity of the colored product is used to calculate in proportion to the amount of HIF-1 α in the original specimen.

Materials provided with the kits:

| | |
|--------------------------------------|-------------------------|
| Reagent | 96/48 Test Kit |
| HIF-1 α Antibody-Coated Wells | 12 strips/6 strips |
| Assay Buffer | 5ml/3ml |
| Standard Diluent | 10ml/5ml |
| HIF-1 α Standard | 2/1vial(s) |
| 50xHIF-1 α Detection Antibody | 210 μ l/105 μ l |
| Detection Antibody Diluent | 10ml/5ml |
| Streptavidin-HRP | 10ml/5ml |
| Wash Buffer Concentrate 20x | 30ml/15ml |
| TMB | 10ml/5ml |
| Stop Solution | 5ml/3ml |
| Plate Covers | 3/2 |

Specimen Collection

- Collecting specimen as following:
 - The particulate of the cell culture supernatants should be removed before use.
 - Serum was obtained from clot at room temperature.



- C. Please collect plasma with EDTA.
D. Assay immediately or store samples at -20°C. Avoid free-thaw cycles.
2. Antiseptic and anticoagulant should not appear in Serum samples.
 3. Any particulate should be removed from samples before use.
 4. Do not use grossly hemolyzed or lipemic samples.
- 3.The Lysis Buffer should contains follow substance for protecting the protein during Lysis cells:
10%(w/v)glycerol,3mM EDTA,1 mM MgCl₂, 20mM β - glycerophosphate, 25mM NaF, 1%TritonX-100 ,25ug/ml Leupeptin, 25ug/ml Pepstatin,and 3ug/ml Aprotinin

Note: Strongly recommend that the serum and plasma samples should be diluent as doubling dilution before use.

Precautions for use:

- 1.Please storage the Kit at 2~8°C。
2. Washing buffer concentrate may have crystal in low temperature, and you can melt its in water-bath before use.
3. Please discard the remains after use of the dissolved standard.
- 4.Avoid contact of substrate solution with oxidizing agents and metal.
- 5.Usage of disposable pipette tips avoid microbial contamination or cross-contamination of reagents or specimens.
6. Do not mix or substitute reagents with those from other lots or other sources.
7. To ensure the adequate mixture of added reagents, please tap gently the plate after the wells were filled with liquid.
8. Incubation temperature should be 25~28°C.
9. Wash step was crucial for whole assay process.
10. Duplicate wells of the same sample were recommended in assay process.
11. Avoid the foam while pour the liquid into wells.
12. For serum or plasma samples ,the biotin-conjugated antibody should be incubate for at least 90 minutes.

Reagent Preparation

- 1.The reagents should be warmed up to room temperature before use. The remanent reagents must reseal and put into refrigeratory again as soon as possible.
- 2.Dilute 1ml of wash buffer Concentrate into 19ml deionized or distilled water to work.
3. If you have a 5x standard diluent, please dilute it with double steaming water or deionized water.
4. Add the standard dilution solution to the bottle according to the volume of the label and wait 15 minutes for complete dissolution. Incubation temperature should be 25~28°C. And in turn add the half concentration diluent by standard diluent .

Wash step:

Automated microplate washer or operating by pipette: Each well should be pour into 300ul wash buffer and soak 15 or 30 seconds,then be aspirated, five times process were repeated. After the last wash, remove remaining wash buffer by aspirating. Invert the plate and blot it against clean paper towels.

Materials Required But Not Provided

1. pipettes and pipette tips
2. Microwell strip reader capable of reading at 450 nm (540 nm as optional reference wave length)
3. automated microplate washer
- 4.Glass-distilled or deionized water

Assay procedure

- 1.The needed strips were putted into the frame, the remains were returned into foil pouch and resealed.
- 2.Blank well were recommended, which only color reagent and stop solution be added. It is suggested that each testing with gradient density of standard for standard curve.
- 3.Add 100ul of standard or sample,. Please diluent 20 or more than 20 times to serum/plasma ,
4. Three times wash process were repeated.
- 5.Add 100ul of detetion antibody.
6. Three times wash process were repeated.
- 7.Add 100ul of Streptavidin-HRP. Cover with the Plate Covers provided. Lucifugal incubation for 30 minutes at room temperature.



8. Three times wash process were repeated.
9. Add 100ul of TMB, Lucifugal incubation for 20 minutes at room temperature.
10. Add 50ul of stop solution to each well, determine the optical density of each well within 10 minutes.

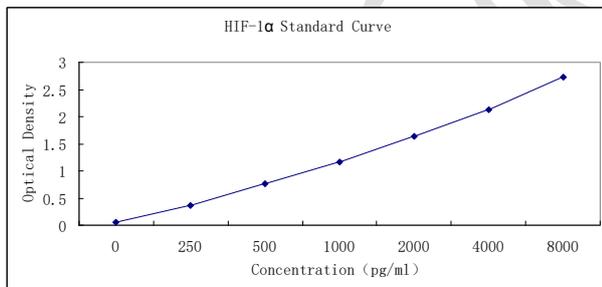
Calculation of Results

1. Duplicates should be within 20 percent of the mean. Average absorbance values for each set of duplicate samples were used as detection results.
2. The blank absorbance values of subtract should be deducted.
3. Drawing a best fit curve through the points of graph. Draw the standard curve by plotting assayed OD value (on the Y axis) vs. concentration (on the X axis). The sample concentration was obtained based on its OD value founding in the standard concentration curve.
4. If the values obtained are not within the expected range of the standard, Samples should be dilute and assay again.

Typical Data and Standard Curve

| concentration (pg/ml) | Typical data 1 | Typical data 2 | Average |
|-----------------------|----------------|----------------|---------|
| 0 | 0.0578 | 0.0524 | 0.0551 |
| 250 | 0.3712 | 0.3618 | 0.3665 |
| 500 | 0.7846 | 0.7264 | 0.7555 |
| 1000 | 1.1924 | 1.1496 | 1.171 |
| 2000 | 1.6382 | 1.6216 | 1.6299 |
| 4000 | 2.1563 | 2.1119 | 2.1341 |
| 8000 | 2.7537 | 2.6955 | 2.7246 |

HIF-1 α Standard Curve



Sensitivity, Specificity, Repeatability

Sensitivity: repeated assays were evaluated and the minimum detectable dose was 43pg/ml.

Specificity : Has no cross-reactivity or interference with HIF-2 α , human HIF-1 α has 100% cross-reactivity or interference with mouse HIF-1 α .

Repeatability: The coefficient of variation between wells or plates is less than 10 percent.

REFERENCES:

1. Carbia-Nagashima A. et al., 2007,Cell 131:309-23.
2. Cho S. et al., 2001, FEBS Lett. 498:62-6.
3. Masson N. et al., 2001,EMBO J. 20:5197-206.
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