人促红细胞生成素(EPO)定量分析
酶联免疫检测试剂盒

本试剂盒仅供科研使用。用于体外定量检测人血清、血浆或细胞培养上清液中的 EPO 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拆，请在收到货一个月之内联系我们。

EPO简介：

促红细胞生成素（EPO）是一个由肾脏产生的激素，能刺激骨髓基质生成红细胞。EPO 是一个糖蛋白，分子量大约为 30000~34000 道尔顿。人 EPO 是 165 个氨基酸的多肽及 1 个 O 位，3 个 N 位连接的糖基有构成的复合物。

组织中的氧浓度的改变决定了肾脏产生 EPO 速率的大小。当组织溶氧浓度较低的时候，就会刺激肾脏产生速率增加，这会引起红细胞的生成的增加。

检测血清中 EPO 的浓度，可作为贫血病、红细胞增多症、发育不全的贫血症、溶血引起的贫血症、缺铁性贫血等病症的辅助诊断指标。

检测原理：

本试剂盒采用双抗体夹心ELISA法检测样本中EPO 的浓度。EPO 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EPO 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人EPO 抗体后，抗人EPO 抗体与EPO 接合，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EPO 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，EPO 浓度与OD_{450}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EPO 浓度。

人EPO定量分析酶联免疫检测试剂盒组成：

组分	规格（96T/48T）
人EPO预包被板	12条/6条
样本分析缓冲液	5ml/3ml
标准品稀释液	10ml/5ml
人EPO标准品	2/1支(冻干)
人EPO生物素化抗体	10ml/5ml
亲和素连接的HRP酶	10ml/5ml
浓缩洗涤液 20×	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：

1.标本的收集请按下列流程进行操作；

A.细胞上清标本离心去除悬浮物后即可；

B.血清标本应是自然凝固后，取上清，避免在冰箱中凝固血液；

C.血浆标本，推荐用EDTA的方法收集

D.若待测样本不能及时检测，标本收集后请分装，冻存于－20℃，避免反复冻融。

2.血清标本不应添加任何防腐剂或抗凝剂；

3.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

4.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。

注：人血清或血浆样本请用样本分析缓冲液做倍比稀释后再检测。

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5×标准品稀释液用双蒸水或去离子水稀释(1份加4份水)。
4.标准品:按标签复溶体积用标准品稀释液复溶使 EPO 终浓度达到 1000pg/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 1000 pg/ml，标准品稀释液直接加入作为 0 浓度.）

洗涤方法:

自动洗板机或人工洗板：每孔洗涤液为300ul，注入与吸出间隔15-30秒。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.建议设置本底较正孔，即空白孔,设置方法为该孔只加 TMB 显色液和中止液。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(100ul/孔)加入相应孔中，用封板胶纸封住反应孔，室温（25-28℃）孵育120分钟。对于血清或血浆标本，请加入50 ul样本分析缓冲液后加50 ul标本，如稀释量大，请将样本与样本分析缓冲液等量加入，不足部分用标准品稀释液补充至100ul。
4.洗板5次，且最后一次置厚吸水纸上拍干。
5.加入生物素化抗体工作液(100ul/孔)。用封板胶纸封住反应孔，室温（25-28℃）孵育60分钟。
6.洗板5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(100ul/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5次，且最后一次置厚吸水纸上拍干。
9.加入显色剂TMB100ul/孔，避光室温（25-28℃）孵育20分钟。
10.加入中止液50ul/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。

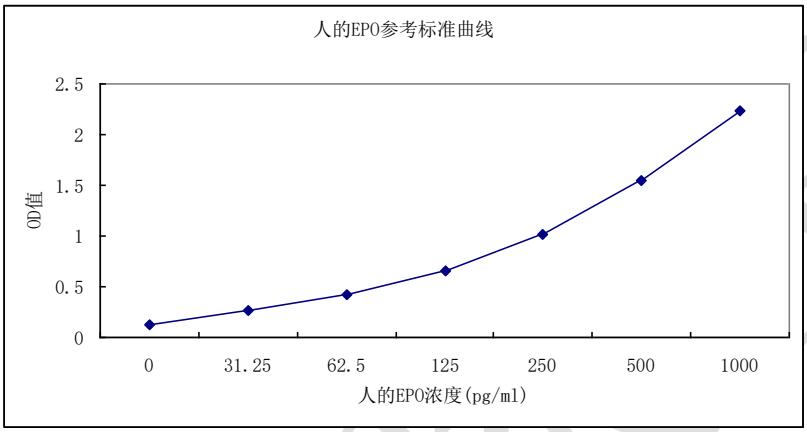
3. 手工绘制标准曲线。以标准品浓度作横坐标，OD值作纵坐标，以平滑线连接各标准品的坐标点。通过标本的OD值可在标准曲线上查出其浓度。

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	0.103	0.145	0.124
31.25	0.235	0.311	0.273
62.5	0.408	0.447	0.4275
125	0.625	0.685	0.655
250	0.988	1.035	1.0115
500	1.511	1.582	1.5465
1000	2.14	2.342	2.241

人EPO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7.8pg/ml。

2.特异性：与人IL-1b , IL-1ra , IL-2, IL-3 , IL-4 , IL-5 , IL-6 , IL-8 ,IL-10,小鼠 EPO没有交叉反应。

3. 重复性：板内，板间变异系数均<10%.

参考文献：

1. Bohling, T. et al. (1987) Erythropoietin in capillary hemangioblastoma in Acta Neuropathol. 74:324.

2. Spivak, J.L. The Mechanism of Action of Erythropoietin. Int J Cell Cloning 1986; 4: 139-166.

3. Jelkmann, W. (1992) Erythropoietin: structure, control of production, and function in Physiol. Reviews 72:449.

4. Spivak, J.L. et al. (1989) Serum immunoreactive erythropoietin in HIV-infected patients in J. Am. Med. Assoc. 261:3104.

ELISA Kit for the Quantitative Analysis of Human EPO

The human EPO ELISA (enzyme-linked immunosorbent assay) kit is used for detection of human EPO in cell culture supernatants,human 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

Erythropoietin (EPO) is a hormone produced by the kidney that promotes the formation of red blood cells in the bone marrow. Erythropoietin (EPO) is a glycosylated protein with a molecular weight of about 30,000 - 34,000 Daltons. Human EPO is a polypeptide consisting of 165 amino acids, containing one O-linked and three N-linked carbohydrate chains [1].

Renal production of Epo is regulated by changes in oxygen availability. Under conditions of hypoxia, the level of Epo in the circulation increases and this leads to increased production of red blood cells.

Quantitation of serum erythropoietin concentration serves as a diagnostic adjunct in determining the cause of anemia or erythrocytosis. Aplastic anemia, hemolytic anemia and anemia due to iron deficiency all result in serum EPO elevation.

Principles of the Test

The kits is a solid sandwich enzyme-linked immunosorbent assay for detection of human EPO. An anti-human EPO monoclonal antibody has been absorbed onto the wells of the microtiter strips provided. Samples including specimens or standards were pipetted into wells. The human EPO in specimens or standards would be captured by the coated antibody and the free others were removed by washing. The human EPO biotin-conjugated antibody were added and binds to human EPO 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, subtrate 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 human EPO in the original specimen.

Materials provided with the kits:

Reagent	96/48Test Kit
Assay Buffer	5ml/3 ml
Human EPO Antibody-Coated Wells	12 strips/6 strips
Standard Diluent	10ml/5ml
Human EPO Standard	2/1vial(s)
Human EPO Detetion Antibody	10ml/5ml
Streptavidin-HRP	10ml/5ml
Wash Buffer Concentrate 20×	30ml/15ml
TMB	10ml/5ml
Stop Solution	5ml/3 ml
Plate Covers	3/2
Complete Instruction Manual	1

Specimen Collection

1. Collecting specimen as following:

A. The particulate of the cell culture supernatants should be removed before use.

B. Serum was obtained from clot at room temperature.

C. Please collect plasma with EDTA.

D. Assay immediately or store samples at -20℃. 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.

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℃。
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 mixure of added reagents, please tap gently the plate after the wells were filled with liquid.
8. Incubation temperature should be 25～28℃.
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 standard diluent to the bottle according to the volume of the label and wait15 minutes for complete dissolution. Incubation temperature should be 25～28℃。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 into300ul 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 ptional 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.Cover with the Plate Covers provided.Incubate for 2 hours at room temperatureIf assay the serum sample,you should add 50μ l assay buffer with 50μ l sample into the wells,if the protein concentration is higher than the range of the Kit, add the same quantitys assay buffer with the sample, the deficiency should be complemented with sample diluent to 100μ l per well.
4.Five times wash process were repeated.
5.Add 100ul of detetion antibody. Cover with the Plate Covers provided.Incubate for 1 hour at room temperature.
6.Five times wash process were repeated.
7.Add 100ul of Streptavidin-HRP. Cover with the Plate Covers provided. Lucifugal incubation for 20 minutes at room temperature.
8.Five times wash process were repeated.
9.Add 100ul of TMB，Lucifugal incubation for 15 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.

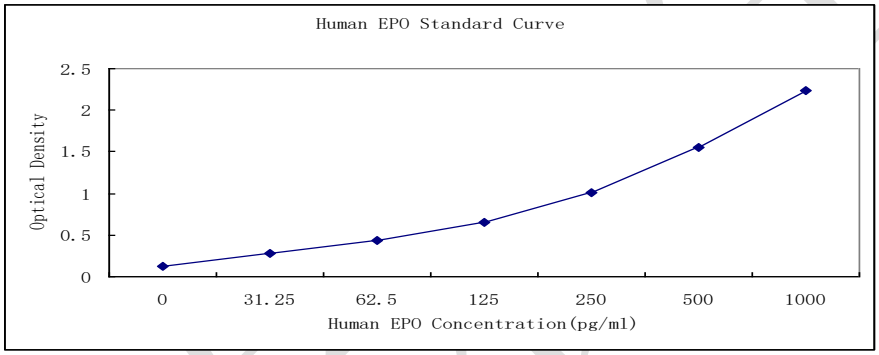
3.Drawing a best fit curve through the points of graph. Draw the standard curve by plotting assayed OD valure (on the Y axis) vs. concentration (on the X axis). The sample concentration was obtained based on its OD value founding in the standard concentration curve.

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	0.103	0.145	0.124
31.25	0.235	0.311	0.273
62.5	0.408	0.447	0.4275
125	0.625	0.685	0.655
250	0.988	1.035	1.0115
500	1.511	1.582	1.5465
1000	2.14	2.342	2.241

Human EPO Standard Curve



Sensitivity,Specificity, Repeatability

Sensitivity: repeated assays were evaluated and the minimum detectable dose was 7.8pg/ml.

Specificity : No significant cross-reactivity or interference with human IL-1b , IL-1ra , IL-2, IL-3 , IL-4 , IL-5 , IL-6 , IL-8 ,IL-10 and Mouse EPO.

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

REFERENCES:

1. Bohling, T. et al. (1987) Erythropoietin in capillary hemangioblastoma in Acta Neuropathol. 74:324.

2. Spivak, J.L. The Mechanism of Action of Erythropoietin. Int J Cell Cloning 1986; 4: 139-166.

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