大鼠胰岛素定量分析酶联免疫检测试剂盒

本试剂盒仅供科研使用。用于体外定量检测大鼠血清、血浆或细胞培养上清液中的胰岛素浓度。**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。

胰岛素简介：

胰岛素是糖代谢中最主要的激素之一。胰腺的β细胞岛细胞产生胰岛素前体蛋白，前体蛋白被加工成C肽和胰岛素。它们以等摩尔浓度进入血循环中。成熟的胰岛素由A、B两条链组成。这两条链是通过两个二硫键桥接形成有功能的胰岛素分子。

血浆葡萄糖浓度的变化是胰岛素产生并分泌的最主要刺激因素，产生的胰岛素具有一些代谢调节作用。其最主要的作用是，将外周血中糖转运到肝脏中贮存起来。一些诸如肝糖生成障碍或在促进血糖升高的激素诸如胰高血糖素、肾上腺素、生长激素和皮质醇等作用下促进肝糖分解都可拮抗胰岛素的作用。

检测原理：

本试剂盒采用双抗体夹心ELISA法检测样本中胰岛素的浓度。胰岛素捕获抗体已预包被于酶标板上，当同时加入标本或参考品和HRP耦连的抗大鼠胰岛素抗体时，其中胰岛素的不同位点会与捕获抗体和HRP耦连的抗大鼠胰岛素抗体结合，形成夹心复合物，锚定在固相载体板上，其它游离的成分通过洗涤的过程被除去。最后加入显色剂，若样本中存在胰岛素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，胰岛素浓度与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，即可算出标本中胰岛素浓度。

大鼠胰岛素定量分析酶联免疫检测试剂盒组成：

组分	规格（96T/48T）
大鼠胰岛素预包被板	12条/6条
标准品稀释液	10ml/5ml
大鼠胰岛素标准品	2支/1支(冻干)*
HRP连接的抗体结合物	10ml/5ml
浓缩洗涤液 20×	30ml/15ml
TMB底物	10ml/5ml
终止液	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的方法收集若待测样本不能及时检测，

D.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，剩余部分请丢弃。

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。

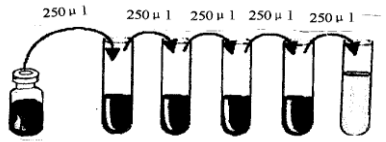
检测前准备工作:

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胰岛素终浓度达到5.5ng/ml，室温反应，请严格控制在25～28℃，静置10~15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5.5ng/ml，标准品稀释液直接加入作为0浓度.）



洗涤方法:

自动洗板机或大鼠工洗板：每孔洗涤液为300ul，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(10ul/孔)加入相应孔中，快速加入HRP连接抗体工作液(100ul/孔)。用封板胶纸封住反应孔，室温（25～28℃）孵育120分钟。

4.洗板5次，且最后一次置厚吸水纸上拍干。

5.加入显色剂TMB100ul/孔，避光室温（25～28℃）孵育20分钟。

6.加入终止液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。

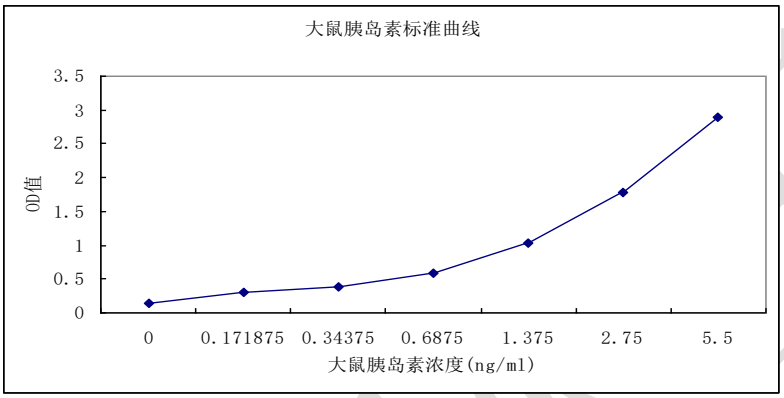
3.手工绘制标准曲线。以标准品浓度作横坐标，OD值作纵坐标，以平滑线连接各标准品的坐标点。通过标本的OD值可在标准曲线上查出其浓度。

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ng/ml	典型OD值1	典型OD值2	OD平均值
0	0.1666	0.1282	0.1474
0.171875	0.3019	0.2871	0.2945
0.34375	0.4281	0.3529	0.3905
0.6875	0.6073	0.5693	0.5883
1.375	1.0635	0.9803	1.0219
2.75	1.8406	1.7362	1.7884
5.5	2.9785	2.8219	2.9002

大鼠胰岛素参考标准曲线



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0.1ng/ml。

2.特异性：不与IGF-I、IGF-II、 小鼠 C肽、大鼠 C肽反应，与猪胰岛素、绵羊胰岛素、小鼠胰岛素、牛胰岛素及人胰岛素分别有628%，256%，75%，110%和195%交叉反应。

3.重复性：板内，板间变异系数均<10%.

参考文献：

Korner J, Savontaus E, Chua SC, Jr., Leibel RL, Wardlaw SL (2001) Leptin regulation of Agrp and Npy mRNA in the rat hypothalamus. J Neuroendocrinol 13:959-966

Olsson R and Carlsson PO (2005) Better vascular engraftment and function in pancreatic islets transplanted without prior culture. Diabetologia 48:469-476

Rydtren T and Sandler S (2002) Efficacy of 1400 W, a novel inhibitor of inducible nitric oxide synthase, in preventing interleukin-1beta-induced suppression of pancreatic islet function in vitro and multiple low-dose streptozotocin-induced diabetes in vivo. Eur J Endocrinol 147:543- 551 10

ELISA Kit for the Quantitative Analysis of Rat Insulin

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

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

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized in the ß-cells of the islets of Langerhans as the precursor, proinsulin, which is processed to form C-peptide and insulin. Both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chains, the A chain and B chain (21 and 30 amino acids respectively). The two chains are linked together by two inter-chain disulphide bridges. Secretion of insulin is mainly controlled by plasma glucose concentration, and the hormone has a number of important metabolic actions. Its principal function is to control the uptake and utilisation of glucose in peripheral tissues via the glucose transporter.

This and other hypoglycaemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycaemic hormones including glucagon, epinephrine (adrenaline), growth hormone and cortisol.

Principles of the Test

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

Materials provided with the kits:

reagent	96/48Test Kit
Rat Insulin Antibody-Coated Wells	12 strips/6 strips
Standard Diluent	10ml/5ml
Rat Insulin Standard	2/1vial(s)
HRP coupled Antibody	10ml/5ml
Wash Buffer Concentrate 20×	30ml/15ml
TMB	10ml/5ml
Stop Solution	5ml/3ml
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 dissolved standard after 3 days for use.

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 the standard dilution solution to the bottle according to the volume of the label and wait15 minutes for complete dissolution. 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 byaspirating.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 10ul of standard or sample then add 100ul of HRP- antibody immediatly.Cover with the Plate Covers provided.Incubate for 120 minutes at room temperature .

4.Five times wash process were repeated..

5. Add 100ul of TMB，Lucifugal incubation for 20 minutes at room temperature.

6. 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 per cent 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

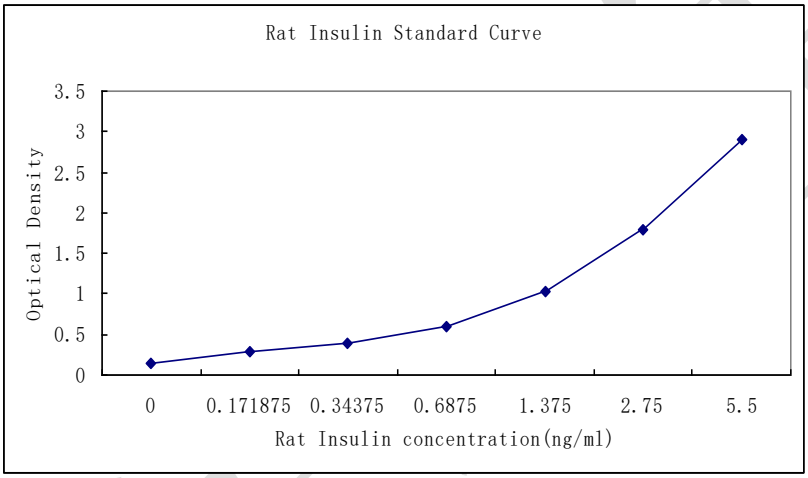
3.Drawing a best fit curve through the points of graph. Draw the standard curve by plotting assayed OD valure (on the Y axis) vs. concentration (on the X axis). The sample concentration was obtained based on its OD value founding in the standard concentration curve.

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 (ng/ml)	Typical data 1	Typical data 2	Average
0	0.1666	0.1282	0.1474
0.171875	0.3019	0.2871	0.2945
0.34375	0.4281	0.3529	0.3905
0.6875	0.6073	0.5693	0.5883
1.375	1.0635	0.9803	1.0219
2.75	1.8406	1.7362	1.7884
5.5	2.9785	2.8219	2.9002

Rat Insulin standard curve



Sensitivity, Specificity, Repeatability

Sensitivity: repeated assays were evaluated and the minimum detectable dose was 0.1ng/ml.

Specificity: No significant cross-reactivity or interference with IGF-I,IGF-II, Mouse C-peptide,Rat C-peptide and has 476% cross-reactivity with porcine insulin, 179% with ovine insulin,75% with mouse insulin ,78% with bovine insulin and 167% with Human insulin.

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

REFERENCES:

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