



**(For scientific research use only, not for clinical diagnosis!)**

## **Rabbit Triiodothyronine (T3) ELISA**

### **Kit Instructions for Use Specifications:**

**48T/96T**

**Please read the instructions carefully before use. If you have any questions,  
please contact us through the following methods: Official hotline: 025-5229-  
8998 Sales department phone: 13914481711 Technical phone: 15950492658  
Contact email: 3224949330@qq.com Company website:  
www.byabscience.cn For specific shelf life, please see the reagents Box  
packaging label. Please use the kit within the shelf life.**

When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.

**Nanjing BYabscience technology Co.,Ltd**

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**Official hotline: 025-5229-8998**

**Supervision phone number:**



**Kit performance Detection range: 0.5**

**nmol/L- 8 nmol/L.**

**Sensitivity:** The lowest detectable dose is less than 0.1 nmol/L.

**Precision:** intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%.

**Recovery rate:** The recovery rate is between 85%-115%.

**Specificity:** This kit recognizes natural and recombinant rabbit triiodothyronine (T3) and has no crossover with structural analogs.

**Stability:** Stored at 2°C-8°C, validity period is 6 months.

**Purpose:** Used to detect the concentration of rabbit triiodothyronine (T3) in samples such as serum, plasma, cell culture supernatant and tissue. **Shelf life:** Stored at 2°C-8°C, valid for 6 months.

## **Experimental principle**

The kit uses enzyme-linked immunoassay method. Biotin-labeled T3 is used, and the purified anti-T3 antibody is coated on the microwell plate. In the competitive inhibition reaction, a certain amount of solid-phase antibody is used to inhibit the competitive reaction with biotin-labeled T3 and non-labeled antigen (calibrator or specimen). The antibody The amount of binding to biotin-labeled T3 is inhibited by the amount of non-labeled antigen. The greater the amount of non-labeled antigen, the less the antibody will bind to biotin-labeled T3, and vice versa. After the reaction is balanced, solid-phase antibody-biotinylation is formed. T3, and then add enzyme-labeled avidin to form a solid-phase antibody-biotinylated T3-enzyme-labeled-avidin complex. After adding substrate for color development, use a microplate reader to measure the absorbance (OD value) at a wavelength of 450 nm. As the T3 concentration increases, the OD value gradually decreases with a good linear relationship. This kit has

the characteristics of high sensitivity, strong specificity, good repeatability, simple and rapid operation, and has reliable detection performance for the reduction or increase of T3 in serum.

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**Kit components and storage: Store unopened kits at 2-8 degrees Celsius. Do not use expired kits.**

Components	48-well configuration	96-well configuration	Store after opening
Pre-coated enzyme plate	48T	96T	<b>2-8°C 14 days</b>
Standard product	0.3mL*6 tubes	0.3mL*6 tubes	<b>2-8°C 14 days</b>
sample diluent	3ml	6ml	2-8°C 180 days
biotinylated antigen	3ml	6ml	<b>2-8°C 14 days</b>
HRP labeled avidin	3ml	6ml	2-8°C 180 days
Chromogenic substrate A	3ml	6ml	2-8°C 180 days
Chromogenic substrate B	3ml	6ml	2-8°C 180 days
stop solution	3ml	6ml	2-8°C 180 days
<b>20×Lotion</b>	15ml	25ml	2-8°C 180 days
sealing film	2 sheets	2 sheets	
manual	1 serving	1 serving	
Ziplock bag	1	1	

The concentrations of calibrators are: 8, 4, 2, 1, 0.5, 0 nmol/L.

Note: 1: Before use, please check whether the label and quantity of the reagents in the kit are consistent with the table.

2: If the components of the kit need to be used again, please ensure that they have not been contaminated since the last use. 3: If the enzyme plate is not used up in a single time, remember to seal it and store it at 2-8°C.

**Prepare your own test equipment required for the test (not provided, but can assist in**

1) Microplate reader capable of detecting absorbance at 450 nm 2) Pipette, pipette tip, and sample addition tank 3) 37°C incubator or water bath 4) Test tubes, centrifuge tubes, measuring cylinders, etc. for preparing reagents 5)

Distilled water or deionized water Ionized water

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6) Vortex oscillator and microplate oscillator.

Notes: 1) For scientific research use only,  
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2) Use within the validity period marked on the kit. Expired products must not be used.

3) Do not mix with kits or components from other manufacturers. Use the sample diluent provided with the kit.

4) If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then re-measure.

5) Human anti-mouse and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.

6) The test results obtained by other methods are not directly comparable to the test results of this kit.

7) Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

8) 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°C。使用后立即冷藏保存试剂。

9) 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。

10) 消除板底残留的液体和手指印，否则影响 OD 值。

11) 底物显色液应呈无色或很浅的颜色。

12) 避免试剂和标本的交叉污染以免造成错误结果。

13) 在储存和温育时避免强光直接照射。

14) 检测使用的酶标仪需要安装能检测 450±10nm 波长的滤光片，光密度范围在 0-3.5 之间。建议使用时提前 15 分钟预热。

15) 试验中所用的 EP 管和吸头均为一次性使用，严禁混用。

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## 样品的准备和保存

以下只是列出样品采集和保存的一般指南。所有样本采集保存过程中，不得使用叠氮钠做为防腐剂。样品如果不立即分析，应分装后冷冻保存，且避免反复冻融。

细胞培养上清——离心去除沉淀，立即分析或分装后-20℃冷冻保存。

血清——用干净试管收集血液，室温凝固 30 分钟，离心 2000×g 20 分钟，收集血清。立即分析或分装后-20℃冷冻保存。

血浆——采用肝素、柠檬酸盐或 EDTA 抗凝，抽血后 30 分钟内在 2-8℃离心 2000×g 20 分钟。为消除血小板的影响，建议在 2-8℃进一步离心 10000×g 10 分钟。立即分析或分装后-20℃冷冻保存。

细胞裂解液——对于贴壁细胞，去除培养液，用 PBS、生理盐水或无血清培养液洗一遍。加入适量裂解液，用枪吹打数下，使裂解液和细胞充分接触。通常 10 秒后，细胞就会被裂解。对于悬浮细胞，离心收集细胞，用 PBS、生理盐水或无血清培养液洗一遍。加入适量裂解液，用枪吹打把细胞吹散，用手指轻弹以充分裂解细胞。充分裂解后，10000—14000×g 离心 3-5 分钟，取上清。立即分析或分装后-20℃冷冻保存。

**Tissue homogenate - rinse the tissue with pre-cooled PBS (0.01M, pH=7.4) to remove residual blood (lysed red blood cells in the homogenate will affect the measurement results), weigh and cut the tissue into pieces. Mix the minced tissue with the corresponding volume of PBS (generally at a weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS. The specific volume can be adjusted appropriately according to experimental needs and recorded. It is recommended to add Protease inhibitor) was added to a glass homogenizer and ground thoroughly on ice. In order to further lyse tissue cells, the homogenate can be sonicated or repeatedly frozen and thawed. Finally, centrifuge the homogenate at 5000 × g for 5 to 10 minutes, and take the supernatant for detection.**

**Urine - Collect in sterile tubes and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If a precipitate forms, centrifuge again.**

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**Reagent preparation 1. Before use, all components must be rewarmed**

**for at least 60 minutes to ensure sufficient rewarming to room**

**temperature.**

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the crystals. Concentrated detergent and distilled water, dilute 1:20, that is, 1 part of concentrated detergent, add 19 parts of distilled water.

**Operating procedures: Return all reagents and components to room temperature first. It is recommended to do duplicate holes for standards, quality control materials and samples.**

1. Prepare the working solution of various components of the kit according to the method described in the previous instructions.
2. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.
3. Take out the pre-coated plate from the sealed bag and set up a blank control well without adding any liquid; set up 2 wells for each calibrator and add 50  $\mu$ l of the corresponding calibrator into each well; add the serum to be tested directly to each of the remaining detection holes. Or 50 $\mu$ l of quality control product.
4. Add 50  $\mu$ l of biotinylated antigen to all wells except the blank well, mix well, attach sealing film, and incubate at 37°C for 60 minutes.
5. Manual plate washing: discard the liquid in the wells, fill each well with washing solution, let stand for 10 seconds and spin dry, repeat 3 times and pat dry. Wash the plate with a plate washer: select the washing program 3 times and pat dry after washing the plate.

**(Tip: In order to obtain ideal experimental results, the residual liquid must be completely removed. After washing the plate, please proceed to the next step immediately and do not let the microplate dry.) 6. Add 50  $\mu$ l of enzyme-labeled avidin to each well (blank (Excluding control wells), mix well, affix sealing film, and incubate at 37°C for 30 minutes.**

7. Manual plate washing: discard the liquid in the wells, fill each well with washing solution, let stand for 10 seconds and spin dry, repeat 3 times and pat dry. Wash the plate with a plate washer: select the washing program 3 times and pat dry after washing the plate.

8、每孔加显色剂 A 50 $\mu$ l，显色剂 B 50 $\mu$ l，振荡混匀后，置 37°C 避光显色 15 分钟，每孔加终止液 50 $\mu$ l。

9、用酶标仪读数，取波长 450nm，先用空白对照孔调零点，然后测定各孔光密度值（OD 值）。

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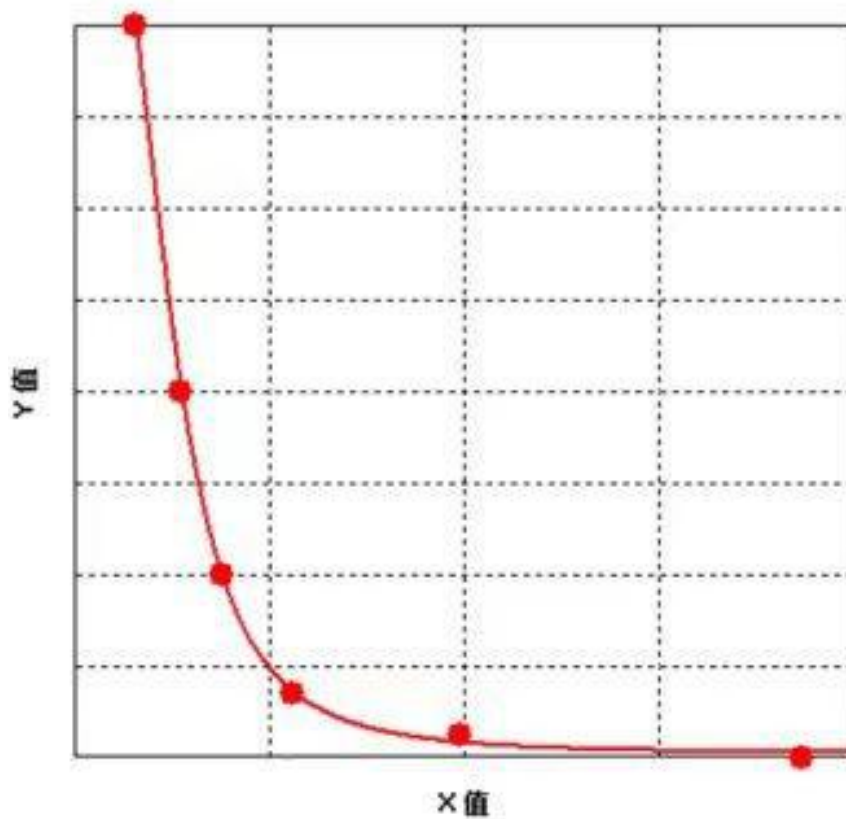
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## 结果计算

9、以标准品浓度做为横坐标，对应的吸光度（OD 值）作为纵坐标，利用计算机软件，采用四参数 Logistic 曲线拟合（4-p1），创建标准曲线方程，通过样品的吸光度（OD 值），利用方程计算样品的浓度值。【用 ELISA Calc 软件计算】10、如果样品被稀释，通过上述方法测的的浓度值，要乘以稀释倍数，才是样品的最终浓度。注意：实验者需根据自己的实验建立标准曲线。每次检测，每块酶标板都必须设立标准曲线。以下曲线仅供参考！



（标曲示意图，仅供参考）

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[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: [问题解答]

问题描述	可能原因	相应对策相应对策
标准曲线梯度差	吸液或加液不准	检查移液器及吸头
	平衡时间太短	保证充足的平衡时间
	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	Improper storage of ELISA kits	Store relevant reagents according to
	Not terminated before reading	Stop solution should be added to

