Fish Tri-iodothyronine(T3) ELISA Kit

Catalog No. CSB-E08488f

(96 tests)

- This immunoassay kit allows for the in vitro quantitative determination of **Fish T3** concentrations in **serum, plasma** and other biological fluids.
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

PRINCIPLE OF THE ASSAY

This assay employs the direct competitive inhibition enzyme immunoassay technique. An antibody specific for T3 has been pre-coated onto a microplate. Add T3 (Standards or samples) to the well, and then add Biotin-conjugated T3. A competitive inhibition reaction is launched between T3 (Standards or samples) and Biotin conjugated T3 with the T3 antibody. Then add the HRP-avidin to each well. The substrate solutions are added to the wells, respectively. And the color develops in opposite to the amount of T3 bound in the initial step. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

0.5 ng/ml-8 ng/ml. The standard curve concentrations used for the ELISA's were 8 ng/ml, 4 ng/ml, 2 ng/ml, 1 ng/ml, 0.5 ng/ml.

SPECIFICITY

This assay recognizes T3. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of T3 is typically less than 0.25 ng/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate (96 tests)	1
Standard	5 x 1 ml
Conjugate	1 x 6 ml
HRP-Avidin	1 x 6 ml
Wash Buffer	1 x15 ml (20×concentrate)
Substrate A	1 x 6 ml
Substrate B	1 x 6 ml
Stop Solution	1 x 6 ml

Standard	S1	S2	S3	S4	S5
Concentration （ng/ml）	0.5	1.0	2.0	4.0	8.0

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 15 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 300 ml of Wash Buffer..

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.

1. Set a Blank well without any solutions.
2. Add 50µl of **Standard** or **Sample** to per well.
3. Add 50µl of **Conjugate** to each well (not to the Blank!).
4. Cover with the adhesive strip. Incubate for 60 minutes at 37° C.

5. Aspirate each well and wash, repeating the process for a total of three to five washes. Wash by filling each well with Wash Buffer (about 200µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 50µl of **HRP-avidin** to each well. Cover with the adhesive strip. Incubate for 30 minutes at 37°C.
7. Aspirate each well and wash as before.
8. Add 50µl of **Substrate A** and 50µl **Substrate B** to each well. Incubate for 10-30 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
9. Add 50µl of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and divide the average zero standard optical density. Create a standard curve by reducing the data using computer software. As an alternative, construct a standard curve by plotting the absorbance ratio for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve

through the points on the graph. The data may be linearized by plotting the log of the T3 concentrations versus the ratio and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.

- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

鱼三碘甲状腺原氨酸(T3)快速检测试剂盒

使用说明书

本试剂盒仅供研究使用

产品编号： CSB-E08488f

检测范围： 0.5 ng/ml-8 ng/ml

最低检测限： 0.25 ng/ml

特异性： 本试剂盒可检测鱼 T3，且与其他相关物质无交叉反应。

有效期： 6 个月

预期应用： ELISA 法定量测定鱼血清、血浆或其它相关生物液体中 T3 含量。

说明

1. 试剂盒保存：2-8℃。
2. 浓洗涤液低温保存会有盐析出，稀释时可在水浴中加温助溶。
3. 中、英文说明书可能会有不一致之处，请以英文说明书为准。
4. 刚开启的酶联板孔中可能会含有少许水样物质，此为正常现象，不会对实验结果造成任何影响。

实验原理

本试剂盒采用酶联免疫直接竞争法检测 T3。微孔板上包被有 T3 抗体。加入 T3 标准品或样品，然后加入生物素标记的 T3。样品中的 T3、生物素标记 T3 与固相板上的抗 T3 抗体发生竞争结合。再向微孔中加辣根过氧化物酶标记的亲和素，形成固相抗体-生物素化 T3-亲和素-HRP 复合物。经加底物显色后，随着样品中 T3 浓度的升高，显色 OD 值呈逐渐下降的线性关系。

试剂盒组成及试剂配制

1. **酶联板(Assay plate)**：一块（96 孔）。
2. **标准品（Standard）**：5×1ml/瓶。

标准品	S1	S2	S3	S4	S5
浓度（ng/ml）	0.5	1.0	2.0	4.0	8.0

3. **生物素标记 T3(Conjugate)**：1×6ml/瓶。
4. **辣根过氧化物酶标记亲和素（HRP-Avidin）**：1×6ml/瓶。
5. **底物溶液 A（Substrate A）**：1×6ml/瓶。
6. **底物溶液 B（Substrate B）**：1×6ml/瓶。
7. **浓洗涤液（Wash Buffer）**：1×15ml/瓶，使用时每瓶用蒸馏水稀释 20 倍。
8. **终止液（Stop Solution）**：1×6ml/瓶。

需要而未提供的试剂和器材

1. 标准规格酶标仪
2. 高速离心机
3. 电热恒温培养箱
4. 超声清洗器
5. 干净的试管和 Eppendof 管
6. 系列可调节移液器及吸头，一次检测样品较多时，最好用多通道移液器
7. 蒸馏水，容量瓶等

浓洗涤液稀释原则：

临用前用蒸馏水进行 20 倍稀释。如有盐析出，稀释后水浴加温助溶。

操作步骤

实验开始前，请提前配置好所有试剂，试剂或样品稀释时，均需混匀，混匀时尽量避免起泡。每次检测都应该做标准曲线。如样品浓度过高时，先进行稀释，以使样品符合试剂盒的检测范围。

1. 加样：分别设空白孔、标准孔、待测样品孔。空白孔不加任何溶液，余孔分别加标准品或待测样品 50ul，注意不要有气泡，加样将样品加于酶标板孔底部，然后在所有孔中加入 50 ul **生物素标记 T3**（空白孔中不加）。尽量不触及孔壁，轻轻晃动混匀。酶标板加上盖或覆膜，37℃反应 60 分钟（为保证实验结果有效性，每次实验请使用新的标准品溶液）。
2. 温育后，弃去孔内液体，甩干，洗板 3-5 次，每次浸泡 10 秒，约 200ul/每孔，甩干。
3. 所有孔中加入 50 ul **辣根过氧化物酶标记亲和素**。尽量不触及孔壁，轻轻晃动混匀。酶标板加上盖或覆膜，37℃反应 30 分钟
4. 按步骤 2 进行洗涤。
5. 依序每孔加**底物溶液 A** 和 **B** 各 50ul，37℃避光显色（30 分钟内，一般 10-15 分钟内，此时肉眼可见标准品的后 3-4 孔有明显的梯度蓝色，前 3-4 孔颜色不明显，即可终止）。
6. 依序每孔加**终止溶液** 50ul，终止反应（此时蓝色立转黄色）。终止液的加入顺序应尽量与底物液的加入顺序相同。为了保证实验结果的准确性，底物反应时间到后应尽快加入终止液。
7. 用酶联仪在 450nm 波长依序测量各孔的光密度（OD 值）。 在加终止液后 15 分钟以内进行检测。

实验备注

1. 用户在初次使用试剂盒时，应将各种试剂管离心数分钟，以便试剂集中到管底。
2. 每次实验留一孔作为空白调零孔，该孔不加任何试剂，只是最后加底物溶液及终止液。测量时先用此孔调 OD 值至零。
3. 为防止样品蒸发，试验时将反应板放于铺有湿布的密闭盒内，酶标板加上盖或覆膜。
4. 未使用完的酶标板或者试剂，请于 2-8℃保存。
5. 建议检测样品时均设双孔测定，以保证检测结果的准确性。

洗板方法

手工洗板方法：吸去（不可触及板壁）或甩掉酶标板内的液体；在实验台上铺垫几层吸水纸，酶标板朝下用力拍几次；将推荐的洗涤缓冲液至少 0.3ml 注入孔内，浸泡 1-2 分钟。根据需要，重复此过程数次。

自动洗板：如果有自动洗板机，应在熟练使用后再用到正式实验过程中。

计算

以标准物的浓度为横坐标（对数坐标），OD 值为纵坐标（普通坐标），在半对数坐标纸上绘出标准曲线，根据样品的 OD 值由标准曲线查出相应的浓度；再乘以稀释倍数；或用标准物的浓度与 OD 值计算出标准曲线的直线回归方程式，将样品的 OD 值代入方程式，计算出样品浓度，再乘以稀释倍数，即为样品的实际浓度。

注意事项

1. 当混合蛋白溶液时应尽量轻缓，避免起泡。
2. 洗涤过程非常重要，不充分的洗涤易造成假阳性。
3. 一次加样时间最好控制在 5 分钟内，如标本数量多，推荐使用排枪加样。
4. 请每次测定的同时做标准曲线，最好做复孔。
5. 如标本中待测物质含量过高，请先稀释后再测定，计算时请最后乘以稀释倍数。
6. 底物请避光保存。
7. 不要用其它生产厂家的试剂替换试剂盒中的试剂。