Thyroxine (T4) ELISA Kit

Cat #: orb1173295 (manual)

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Lot #: Refer to product label

Detection range: 20 ng/mL-320 ng/mL

Sensitivity: 10 ng/mL

Precision: Intra-assay Precision: The CV (%) < 15%. Inter-assay Precision: The CV (%) <15%

Recovery: The recovery ranged from 85% to 115% with an overall mean recovery of 100%.

Specificity: Biorbyt's Thyroxine (T4) ELISA Kit has high sensitivity and excellent specificity for detection of T4. No significant cross-reactivity or interference between T4 and analogues was observed.

Applicable samples: Serum, Plasma

Storage: The unopened kit should be stored at 4°C for 12 months.

Assay Principle

T4 is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. The level of T4 is decreased in hypothyroid patients and is increased in hyperthyroid patients. The major form of thyroid hormone in the blood is thyroxine (T4), which has a longer half-life than T3. In humans, the ratio of T4 to T3 released into the blood is between 14: 1 and 20: 1. T4 is converted to the active T3 (three to four times more potent than T4) within cells by deiodinases (5'-iodinase). These are further processed by decarboxylation and deiodination to produce iodothyronamine (T1a) and thyronamine (T0a). All three isoforms of the deiodinases are selenium- containing enzymes, thus dietary selenium is essential for T3 production. Edward Calvin Kendall was responsible for the isolation of thyroxine in 1915. Biorbyt's Thyroxine (T4) ELISA Kit employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody specific to T4. Standards or samples are added to the appropriate microtiter plate wells with Biotin-conjugated T4. A competitive inhibition reaction is launched between T4 (Standards or samples) and Biotin-conjugated T4 with the pre-coated antibody specific forT4. The more amount of T4 in samples, the less antibody bound by Biotin-conjugated T4. After washing, Avidin-HRP is added to the wells. Substrate solution is added to the wells and the color develops in opposite to the amount of T4 in the sample. The color development is stopped and the intensity of the color is measured.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
T4 Standard	0.5 mL×5	1 mL×5	4℃
Avidin-HRP	3 mL	6 mL	4℃
Biotin conjugated T4	3 mL	6 mL	4℃
HRP substrate A	3.5 mL	7 mL	4℃, protected from light
HRP substrate B	3.5 mL	7 mL	4℃, protected from light
Stop solution	3.5 mL	7 mL	4℃
Wash buffer (20×)	7.5 mL	15 mL	4℃
T4 microplate	48 wells	96 wells	4℃
Plate covers	1	2	RT

Note: Std1: 20 ng/mL; Std2: 40 ng/mL; Std3: 80 ng/mL; Std4: 160 ng/mL; Std5: 320 ng/mL.

Materials Required but Not Supplied

- Microplate Reader capable of measuring absorbance at 450 nm
- Multi channel pipetteor automated microplate washer
- Incubator, Refrigerated Centrifuge
- Precision Pipettes, Disposable Pipette Tips
- Deionized Water

Reagent Preparation

Note: Bring all reagents equilibrate to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.

1×Wash buffer: Wash buffer (20×) dilute with deionized water 1: 20 to obtain the 1×Wash Buffer. Store at 4℃.

Sample Preparation

1. Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1, 000 g. Remove serum and assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.

2. Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1, 000 g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Note: Do not use grossly hemolyzed or lipemic specimens. If samples are to be used within 24 hours, they may be stored at 2 to 8°C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add 50 µL of T4 Standard or Sample per well. It is recommended that all Standards and Samples be added in duplicate to the microplate. Set a Blank well without any solution.
3. Add 50 µL of Biotin conjugated T4 to each well (not to Blank well). Mix well, cover with the plate cover provided and then incubate for 1 hat 37℃.
4. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well with 1×Wash Buffer (250 µL) using a multi-channel pipette automated microplate washer, and let it stand for 10 s, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.
5. Add 50 µL of Avidin-HRP to each well (not to Blank well), mix well and cover with the plate cover provided. Incubate for 30 min at 37℃.
6. Repeat the wash as in step 4.
7. Add 50 µL of Substrate A and 50 µL of Substrate B to each well, mix well and cover with the plate cover provided. Incubate for 15 min at 37℃. Keeping the plate away from drafts and other temperature fluctuations in the dark.
8. Add 50 µL of Stop solution to each well. Stop Solution should be added to the plate in the same order as HRP substrate. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 30 min, using a microplate reader set to 450 nm.

Data Analysis

1. Average the duplicate readings for each standard and sample.
2. Drawing of standard curve: With the standard solution concentration as the x-axis and the mean absorbance for each standard as they-axis, draw the standard curve. A computer software can be used to create a standard curve.

Typical Data

Typical standard curve（$R^2 \geq 0.99$）

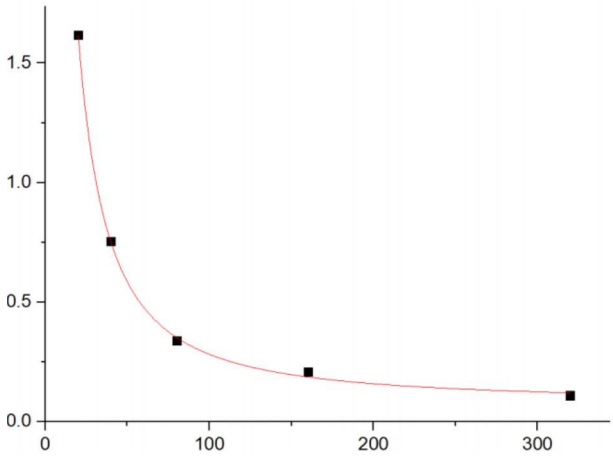

Figure1. Standard Curve of T4 in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Precautions

1. Do not mix or substitute reagents with those from other lots or sources.
2. 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.
3. To ensure accurate results, proper adhesion of plate covers during incubation steps is necessary.
4. Stop Solution has certain Corrosive. Please take protective measures when operating.