General Triiodothyronine ELISA Kit (T3)

Catalog NO.: RK00719

version: 2.0

This package insert must be read in its entirety before using this product

Introduction

This ELISA kit used for quantitative determination of T3 in serum, plasma, cell culture supernatants, cell lysates, tissue homogenates and other biological fluids. For research use only, and it is highly recommended to read thoroughly of this manual before using the product.

Principle of the Assay

This assay is based on the competitive binding technique in which T3 present in a sample competes with a fixed amount of horseradish peroxidase (HRP)-labeled T3 for sites on a mouse monoclonal antibody. Following a wash to remove excess conjugate and unbound sample, a chromogenic substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped by acid, and the absorbance is read at 450 nm. The intensity of the color is inversely proportional to the concentration of Cortisol in the sample.

Material Provided & Storage Conditions

Unopened kits can be stored at 2-8° C for 1 year, and opened products must be used within 1 month.

Part	Size	Cat. No.	Storage of opened/reconstituted material
Antibody Coated Plate	8×12	RM03537	Put the unused slats back in the aluminum foil bag with the desiccant and reseal them. They can be stored at 2-8° C for 1 month.
Standard Lyophilized	2 vials	RM03538	It is not recommended to use again after redissolving.
Concentrated HRP-Conjugate Antigen(100×)	1 ×60μL	RM03539	Store at 2-8° c for 1 month *
Standard/Sample Diluent (R1)	1 ×20mL	RM00023	Store at 2-8° c for 1 month

HRP- Conjugate Antigen Diluent(R2)	1 ×12mL	RM00024	*
Wash Buffer(20x)	1 ×30mL	RM00026	
TMB Substrate	1 ×12mL	RM00027	
Stop Solution	1 ×6mL	RM00028	
Plate Sealers	4 Strips		
Specification	1		

Other Supplies Required

1. Microplate reader capable of measuring absorbance at 450

nm, with the correction wavelength set at 630 nm or 570 nm.

2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. Incubator.
6. Test tubes for dilution of standards and samples.

Precautions

* FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC

PROCEDURES.

1. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
2. Variations in sample collection, processing, and storage may cause sample value differences.
3. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
4. Stop Solution contains strong acid. Wear eye, hand, and face protection.
5. Please perform simple centrifugation to collect the liquid before use.
6. Do not mix or substitute reagents with those from other lots or other sources.
7. Adequate mixing is particularly important for good result. Use a mini-vortexer at the lowest frequency.
8. Mix the sample and all components in the kits adequately and use clean plastic container to prepare all diluents.
9. Both the sample and standard should be assayed in duplicate, and reagents should be added in sequence in accordance with the requirement of the specification.
10. Reuse of dissolved standard is not recommended.

11. The kit should not be used beyond the expiration date on the kit label.
12. The kit should be away from light when it is stored or incubated.
13. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma, and other biological fluids in accordance with NCCLS regulations.
14. To avoid cross contamination, please use disposable pipette tips.
15. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
16. This assay is designed to eliminate interference by other factors present in biological samples.
17. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.
18. The 48T kit is also suitable for the specification.

Sample Collection & Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Samples containing the correlated IgG as in this kit may interfere with this assay.

Cell Culture Supernatant: Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 ° C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20 ° C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles. (Note: Citrate plasma has not been validated for use in this assay.)

Cell Lysates : Cells need to be lysed before assaying according

to the following directions. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000xg for 5 minutes (suspension cells can be collected by centrifugation directly). Wash cells three times in cold PBS. Resuspend cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified. Centrifuge at 1,500×g for 10 minutes at 2-8 ℃ to remove cellular debris. Assay immediately or aliquot and store at ≤-20 ℃.

Tissue homogenates: The preparation of tissue homogenates varies depending upon tissue type. Tissues are rinsed in ice-cold PBS to remove excess blood thoroughly and weigh before homogenization. Mince the tissues to small pieces and homogenized them in fresh lysis buffer with a glass homogenizer on ice or using Micro Tissue Grinders. Different lysis buffer should be chosen based on subcellular location of the target protein (e.g. 1mL lysis buffer is added in 200mg tissue sample). The resulting suspension is sonicated with an ultrasonic cell disrupter till the solution is clarified. Then, the homogenates are centrifuged for 5 minutes at 10,000 × g. Collect the supernatants and assay immediately or aliquot and store at ≤

-20 ° C.

Other biological fluids: Centrifuge samples for 20 minutes at 1,000×g. Collect the supernatants and assay immediately or store samples in aliquot at -20° C or -80° C for later use. Avoid repeated freeze-thaw cycles.

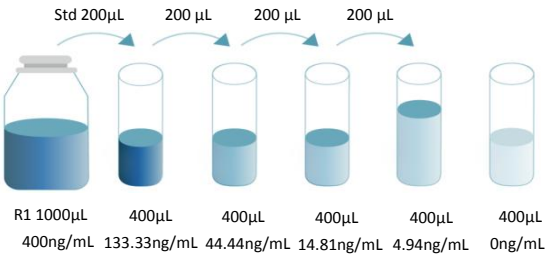
Note: It is suggested that all samples in one experiment be collected at the same time of the day. Avoid hemolytic and hyperlipidemia sample for serum and plasma.

Reagent Preparation

Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.

Standard - Reconstitute the Standard Lyophilized with 1.0 mL Standard/Sample Diluent(R1). This reconstitution produces a stock solution of 400ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use the 400ng/mL standard stock to produce a dilution series (below) with Standard/Sample Diluent(R1). Mix each tube thoroughly and change pipette tips between each transfer (recommended concentration for standard curve: 400, 133.33, 44.44, 14.81, 4.94, 0ng/mL). Use diluted standards within 60 minutes of preparation.



Working HPR-Conjugate Antigen - Dilute 1:100 of Concentrated HRP-Conjugate Antigen (100x) with HRP-Conjugate Antigen Diluent (R2) before use. For example: Add 10 µL of Concentrated HRP-Conjugate Antigen (100x) to 990 µL HRP-Conjugate Antigen Diluent (R2) to prepare 1000 µL Working HRP-Conjugate Antigen Buffer.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have

completely dissolved. Dilute 1:20 with double distilled or deionized water before use, for example：Add 20 mL of Wash Buffer Concentrate to 380 mL of deionized or distilled water to prepare 400 mL of Wash Buffer.

Assay Procedure

Bring all reagents and samples to room temperature before use.

It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL Standard/sample Diluent (R1) in a blank well.
4. Add 50 µL different concentration of standard or sample in other wells. Record the plate layout of standards and sample assay.
5. Add 50 µL Working HRP-Conjugate Antigen in each wells, and cover with new adhesive sealer provided.
6. Incubate for 1.5 hour at 37° C on a horizontal orbital microplate shaker (0.12″ orbit) set at 500 ± 50 rpm. During the incubation, turn on the microplate reader to warm up.
7. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 350 µL Wash Buffer using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for 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.

8. Add 100 μL TMB Substrate to each well. Incubate for 15-20 minutes at 37° C. Protect from light.

9. Add Stop Solution (50 μL/well), determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may cause higher value and less accurate result.

Assay Procedure Summary

Prepare the standard and reagents

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Add 50ul of standards or test samples to each well

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Add 50ul Working HRP-Conjugate Antigen

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Incubate for 1.5 hours at 37° C, then wash 5 times

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Add 100ul Substrate Solution

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Incubate for 15-20 min at 37° C under dark condition

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Add 50ul Stop Solution

↓

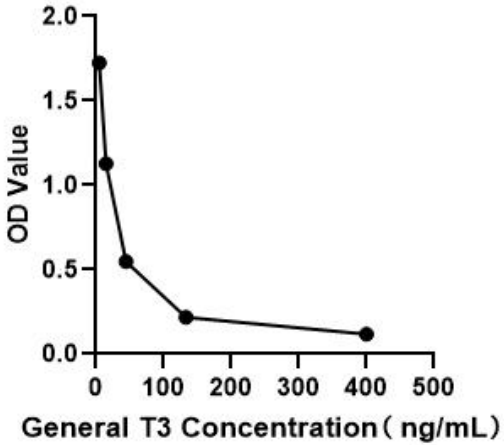
Detect the optical density within 5 minutes under 450nm.

Correction Wavelength set at 570nm or 630nm

Calculation of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. Create a standard curve by reducing the data using computer software capable of generating a four- parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the T3 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of T3 assayed.

Detection Range

4.94-400ng/mL

Sensitivity

The minimum detectable dose (MDD) of T3 typically less than 1.65ng/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This method has high sensitivity and specificity for T3 detection, and there is no obvious cross-reaction or interference between T3 and analogues.

Note:

Due to the limitations of existing technology and knowledge, it is impossible for us to complete the cross-reaction detection between T3 and all analogues, so the cross-reaction may still exist.

Precision

Intra-plate Precision

3 samples with low, middle and high level T3 were tested 20 times on one plate, respectively.

Intra-Assay: CV<10%

Inter-plate Precision

3 samples with low, middle and high level T3 were tested on 3 different plates, 20 replicates in each plate.

Inter-Assay: CV<15%

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(ng/mL)	50	200	350	150	250	400
Standard deviation	2.15	9.4	16.45	13.5	14.5	31.6
CV(%)	4.3	4.7	4.7	9	5.8	7.9

Recovery

Matrices listed below were spiked with certain level of T3 and the recovery rates were calculated by comparing the measured value to the expected amount of T3 in samples.

Sample	Average Recovery (%)	Range (%)
Cell Culture Media(n=5)	93	83-103
Serum(n=5)	89	81-97

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of T3 and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

/	/	Cell Culture Media(n=5)	Serum(n=5)
1:2	Average of Expected (%)	92	87
	Range (%)	85-99	80-94
1:4	Average of Expected (%)	98	95
	Range (%)	92-104	87-103
1:8	Average of Expected (%)	100	91
	Range (%)	95-105	83-99
1:16	Average of Expected (%)	95	92
	Range (%)	90-100	84-100

Trouble Shooting

Problem	Possible Cause	Solution
High Background	Insufficient washing	Sufficiently wash plates as required. Ensure appropriate duration and number of washes. Ensure appropriate volume of wash buffer in each well.
	Incorrect incubation procedure	Check whether the duration and temperature of incubation are set up as required.
	Cross-contamination of samples and reagents	Be careful of the operations that could cause cross-contamination. Use fresh reagents and repeat the tests.
No signal or weak signal	Incorrect use of reagents	Check the concentration and dilution ratio of reagents. Make sure to use reagents in proper order.
	Incorrect use of microplate reader	Warm the reader up before use. Make sure to set up appropriate main wavelength and correction wavelength.
	Insufficient colour reaction time	Optimum duration of colour reaction should be limited to 15-25 minutes.
	Read too late after stopping the colour reaction	Read the plate in 5 minutes after stopping the reaction.

	Matrix effect of samples	Use positive control.
Too much signal	Contamination of TMB substrate	Check if TMB substrate solution turns blue. Use new TMB substrate solution.
	Plate sealers reused	Use a fresh new sealer in each step of experiments.
	Protein concentration in sample is too high	Do pre-test and dilute samples in optimum dilution ratio.
Poor Duplicates	Uneven addition of samples	Check the pipette. Periodically calibrate the pipette.
	Impurities and precipitates in samples	Centrifuge samples before use.
	Inadequate mixing of reagents	Mix all samples and reagents well before loading.

*For research purposes only. Not for therapeutic or diagnostic purposes.