Thyroxine (T4)

ELISA kit (Serum & Plasma)

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-55116-96

www.zellx.de

Sample Types Validated for:

Serum, EDTA and Heparin Plasma

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

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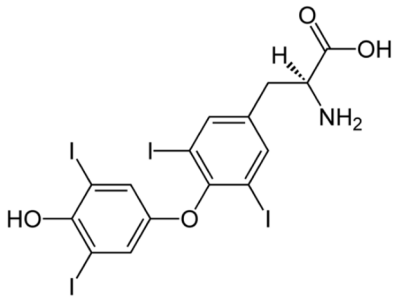
Please read this insert completely prior to using the product.

Introduction

Background

Thyroid hormones produced and released by thyroid gland are tyrosine-based hormones and consist of two hormones: Thyroxine (T4) which contains 4 atoms of iodine, and triiodothyronine (T3), which has 3 atoms of iodine. These hormones are primarily responsible for regulation of metabolism. Due to their structure deficiency in iodine leads to decreased production of T3 and T4, which enlarges the thyroid tissue and will cause the disease named goiter. Thyroxine is the main hormone produced by the thyroid gland and the major form of thyroid hormone in the blood (The blood ratio of T4 to T3 is approximately 20 to 1). The hypothalamus and the pituitary gland (located in the brain) are involved in controlling the thyroid gland and its hormones via Thyroid stimulating hormone (TSH) modulation. Thyroid hormones are very critical for the human body and regulate some of developmental, metabolic, and neural activities in the body. Approximately 20% of T3 is produced in the thyroid gland; the rest of T3 however, is generated by the deiodination of T4 in peripheral tissues. Although circulating levels of T4 are much higher than T3 levels, T3 is more metabolically active (3-4 times more than T4).

Circulating thyroid hormones are mostly bound to carrier proteins (e.g. thyroid- binding globulin [TBG], prealbumin and albumin); the biologically active form of T3 is however, the unbound (free) T3. Although both T3 and T4 are bound to TBG, T4 is bound more firmly than T3. Under-production of thyroxine by the thyroid gland leads to hypothyroidism. This condition is either due to naturally underactivity of thyroid gland or because of radioiodine therapy or surgery for an overactive gland.



Assay principle

The ZellX® Thyroxine (T4) Immunoassay kit is a competitive ELISA designed to quantitatively measure T4 present in serum and plasma samples. A T4 stock solution is provided to generate a standard curve for the assay and all samples should be read off the standard curve. This assay has been designed to measure total T4 in extracted serum and plasma.

The kit includes a 96-well plate that is pre-coated with a secondary anti-mouse antibody. The function of this antibody is to capture the mouse anti-T4 antibody bound to T4 conjugate (peroxidase-labeled) and hold this complex to the plate during the subsequent detection steps. The T4-conjugate (labeled) and the sample T4 (unlabeled) compete for binding to the mouse antibody. After 1 hour of incubation, the substrate is added to react with the peroxidase-labeled antibody-antigen conjugate. After stopping the reaction, the intensity of the generated color can be measured at 450 nm. The lower the amount of T4 in the sample, the stronger the signal due to more labeled T4 bound to the well.

General information

Materials supplied in the Kit

Component	*Quantity*
T4 Standard (1 µg/mL)	40 µL
T4 Antibody	2.6 mL
T4 Conjugate	2.6 mL
Assay Buffer Concentrate (5x)	11 mL
Wash Buffer Concentrate (20x)	25 mL
Dissociation Reagent	1 mL
TMB Substrate	11 mL
Stop Solution	5 mL
Coated Clear 96-Well Plate & Sealer	1 plate

Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit.

Materials required but not supplied

Deionized water (diH_2O)

Microplate/ELISA reader capable of reading optical absorption at 450 nm

Microplate shaker, Centrifuge, Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Stop Solution is an acidic solution and should not come in contact with skin or eyes. Handling this reagent needs appropriate precaution.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.

- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- The antibody-coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.
- This kit utilizes a peroxidase-based readout system. Buffers, including Wash Buffers from other manufacturers, containing sodium azide will inhibit color production by the enzyme. Make sure all buffers used for samples are azide-free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer.

Assay protocol

Reagent preparation

i. **Assay Buffer:** Prepare a 1:5 dilution of Assay Buffer Concentrate with diH_2O (1 part Assay Buffer Conc. with 4 parts diH_2O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

ii. **Wash Buffer:** Prepare a 1:20 dilution of Wash Buffer Concentrate with diH_2O (1 part Wash Buffer Conc. with 19 parts diH_2O), and mix well. Assay Buffer can be stored at room temperature for up to 3 months.

Sample preparation

Samples containing visible particulate should be centrifuged prior to conducting the assay. Moderate to severely hemolyzed samples should not be used for this assay.

Since T4 is identical across all species, it is expected that this kit can measure T4 in human and other species.

All samples and standards must be used within 2 hours of preparation or must be stored at ≤ -20 for later analysis.

I. **Serum, Plasma:**
- Collect the fresh serum or plasma with heparin or EDTA.
- Centrifuge at 600 g for 10 min at 4˚C.
- Separate the serum or plasma from the red blood cells, and transfer into fresh tubes.
- Serum and plasma samples need to be treated with the supplied Dissociation Reagent. Adding this reagent will yield the total T4 concentration in serum or plasma.
- Allow the Dissociation Reagent to warm completely to Room Temperature before use.

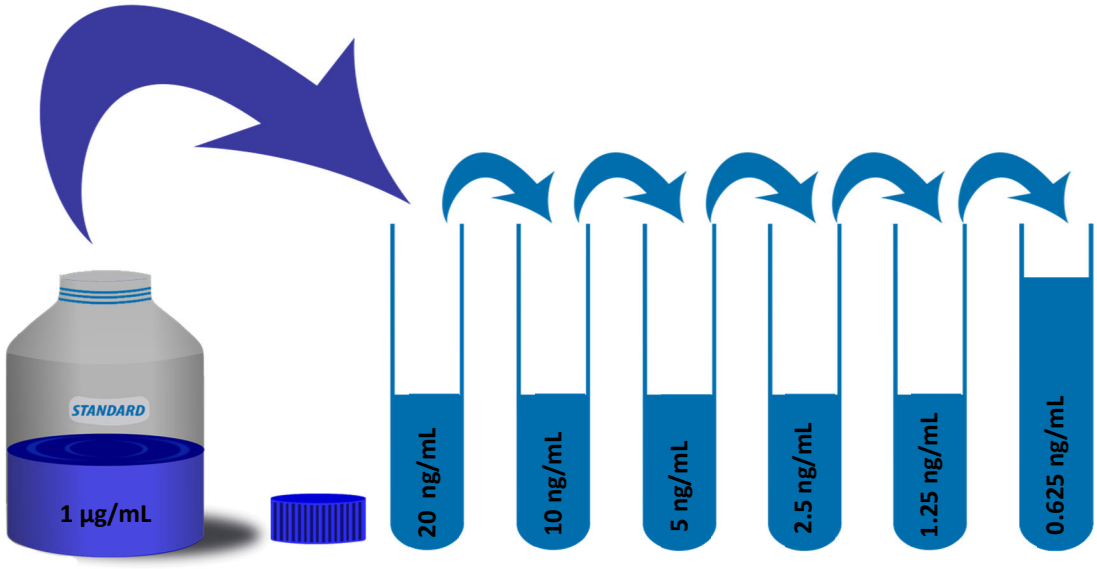
- Add 5 µL of Dissociation Reagent into 1 mL Eppendorf tubes.
- Add 5 µL of serum or plasma to the Dissociation Reagent in the tube, vortex gently and incubate at room temperature for at least 5 minutes.
- Add 90 µL of Assay Buffer to the tube.
- This 1:20 dilution can be diluted further with Assay Buffer. Final serum and plasma dilutions should be ≥ 1:20.

All the samples must be used within 2 hours of preparation; otherwise, aliquots of the sample should be kept at ≤ -20˚C for later use.

Standard preparation

- Prepare a 1:50 dilution of T4 Standard with Assay Buffer (mix 10 µL of standard with 490 µL of Assay Buffer), and label as the Standard No.6 (20 ng/mL).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 pg/mL standard.

No.	*Concentration*	*Material needed*
Standard No.6	20 ng/mL	10 µL T4 Standard + 490 µL Assay Buffer
Standard No.5	10 ng/mL	250 µL Standard No.6 + 250 µL Assay Buffer
Standard No.4	5 ng/mL	250 µL Standard No.5 + 250 µL Assay Buffer
Standard No.3	2.5 ng/mL	250 µL Standard No.4 + 250 µL Assay Buffer
Standard No.2	1.25 ng/mL	250 µL Standard No.3 + 250 µL Assay Buffer
Standard No.1	0.625 ng/mL	250 µL Standard No.2 + 250 µL Assay Buffer
Standard No.0	0 pg/mL	250 µL Assay Buffer



All standard must be used within 2 hours of preparation

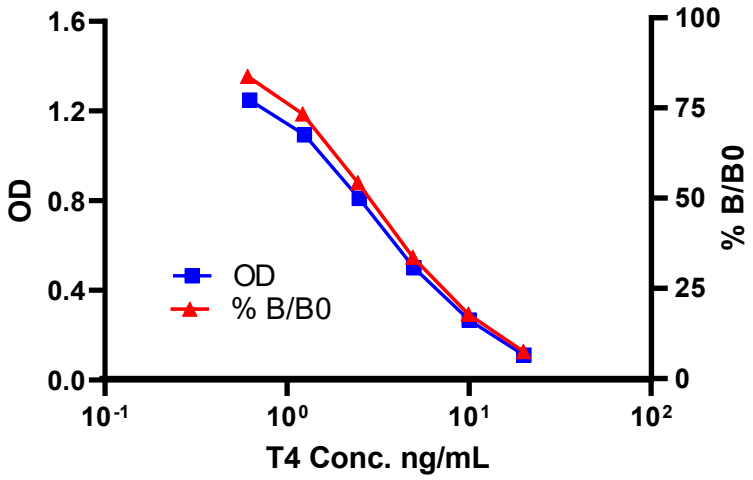
Assay Procedure

1. Pipette 10 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 10 µL of Assay Buffer into duplicate wells of the Zero standard.
3. Pipette 35 µL of Assay Buffer into duplicate wells of the nonspecific binding (NSB).
4. Add 25 µL of T4 Conjugate to each well, using a repeater pipette.
5. Add 25 µL of T4 Antibody to each well except the NSB wells, using a repeater pipette.
6. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
7. Cover the plate with the plate sealer and shake for 1 hours at room temperature. If the plate is not shaken, signals will be approximately 40 % lower.
8. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer.
9. Tap the plate on clean absorbent towels to dry.
10. Add 100 µL of TMB Substrate to each well using a multichannel/repeater pipette.
11. Incubate at room temperature for 30 minutes without shaking.
12. Add 50 µL of Stop Solution to each well using a multichannel/repeater pipette.
13. Read the optical density at 450 nm.

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Subtract the mean ODs of the NSB from all OD values
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader
- Calculate the % B/B0 ratio.
  - **Note:** B0 is the binding for the zero standard or the maximum binding well, which represents the maximum signal from enzyme captured by the specific antibody in competitive ELISA. All other standards and samples are expressed as a percentage of this value (% B/B0).
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

Conversion Factor: 77.7 ng/mL of Thyroxine is equivalent to 100 nM.



A typical standard curve of ZellX® T4 ELISA kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® T4 ELISA kit was determined as 0.80 ng/mL.

Sensitivity

The sensitivity of the ZellX® T4 ELISA kit was determined as 0.23 ng/mL.

Precision

Intra-Assay Precision (Precision within an assay): 2 human samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 2 human samples were tested in duplicate on 18 different assays over multiple days.

Item	*%CV*
Intra assay	3.0, 2.9
Inter assay	6.3, 7.8

Cross Reactivity

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	*Cross Reactivity (%)*
Thyroxine (T4)	100
Reverse T3 (3,3',5'-Triiodo-L-thyronine)	89.0
T3	5.23

Protocol summary