Triiodothyronine (T3)

ELISA kit

(96 Tests)

ZELLX GmbH (Germany)

CAT No. ZX-55115-96

www.zellx.de

Sample Types Validated for:

Extracted Serum and Plasma, Urine, Dried Fecal Extracts, and Tissue Culture Media

!!! 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.

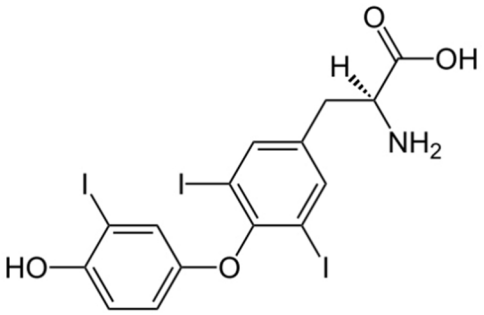
Introduction

Background

Thyroid hormones produced and released by thyroid gland consist of two hormones: Thyroxine, which contains 4 atoms of iodine (T4), and triiodothyronine (T3), which has 3 atoms of iodine. The hypothalamus and the pituitary gland (located in the brain) are involved in controlling the thyroid gland and its hormones. Thyroid hormones are very critical for the human body and regulate some of developmental, metabolic, and neural activities. Approximately 20 percent of T3 is produced in the thyroid gland; the rest of T3 however, is generated by the deiodination of T4 to T3 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), it is worth noting however, due to shorter half-life, T3 effect is briefer.

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. Total T3 consists of both the bound and unbound fractions.

In hyperthyroidism, also known as overactive thyroid, the level of both T4 and T3 are usually increased, however, a small subgroup of the patients shows a pattern (T3 thyrotoxicosis) with elevated free T3 but normal free T4. hypothyroidism patients oppositely showed decreased levels of both T4 and T3.



Assay principle

The ZellX® T3 Immunoassay kit is a competitive ELISA designed to quantitatively measure T3 present in serum, plasma, urine, extracted dried fecal samples, and tissue culture media samples. A T3 stock solution is provided to generate a standard curve for the assay and all samples should be read off the standard curve.

The kit includes a 96-well plate that is pre-coated with a secondary goat anti-sheep antibody. The function of this antibody is to capture the mouse anti-T3 antibody bound to T3 conjugate (peroxidase-labeled) and hold this complex to the plate during the subsequent detection steps. The T3-conjugate (labeled) and the sample T3 (unlabeled) compete for binding to the mouse antibody. After 2 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 T3 in the sample, the stronger the signal due to more labeled T3 bound to the well.

General information

Materials supplied in the Kit

Component	*Quantity*
T3 Standard (200 ng/mL)	70 µL
T3 Antibody	2.6 mL
T3 Conjugate	2.6 mL
Assay Buffer Concentrate (5x)	11 mL
Wash Buffer Concentrate (20x)	25 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 except the **T3 Standard** and **T3 Conjugate** which must store at -20°C upon receipt.

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

For Dried Fecal Sample:

ACS Grade Ethanol

Glass test tubes

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 T3 is identical across all species, it is expected that this kit can measure T3 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:**
   - Dilute the serum and plasma with 5-time volume of the ethyl acetate. (1 volume of serum/plasma with 5 volume of ethyl acetate)

- Mix solutions by vortexing for 2 minutes. Allow layers to separate for 5 minutes.
- Freeze samples in a dry ice/ethanol bath and pipet off the solvent solution from the top of the sample into a clean tube. Repeat last 3 steps for maximum extraction efficiency, combining the solvent solutions.
- Dry pooled solvent extracts down in a speedvac for 2-3 hrs. If samples need to be stored they should be kept at -20°C.
- Re-dissolve samples at room temperature in the Assay Buffer. A minimum of 250 µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.

II. **Urine:**

- Urine should be diluted ≥ 1:4 by taking one part of sample and adding 3 or more parts of Assay Buffer prior to conducting assay.
- **Normalize the sample value based on creatinine levels using our Urine Creatinine assay kit Cat NO. ZX-44110-96 in random urine specimen.**

III. **Dried Fecal Sample:**

- Ensure that the sample is completely dry, and powder the sample to improve extraction recovery. Remove any large particles if possible.
- Weigh out ≥ 0.2 gram of dried fecal solid into a tube. Samples can be dried by passive drying, gentle heating (≤ 60°C), or freeze-drying (lyophilization).
- Add 1 mL of ethanol per 0.1 gram of solid fecal sample (100 mg/mL) and seal.
- Shake strongly for at least 30 minutes.
- Centrifuge at 5000 rpm at 4°C for 15 minutes and collect supernatant in a clean tube. This material can be stored at ≤ -20°C for at least a month if properly sealed.
  - **Note:** Samples containing low levels of analyte can be concentrated by drying down the extract and resuspension in a reduced volume of Assay Buffer.
- Supernatant should be diluted ≥ 1:5 by taking one part of sample and adding 4 or more parts of Assay Buffer.
- Vortex well and allow to rest 5 minutes at room temperature
- Repeat the vortex step 2 more times to ensure complete steroid solubility.
- The final concentration of ethanol in the sample to be added to the wells should be ≤ 5%. **(≥ 1:4 dilution with Assay Buffer is needed.)**

IV. **Tissue Culture Media:**

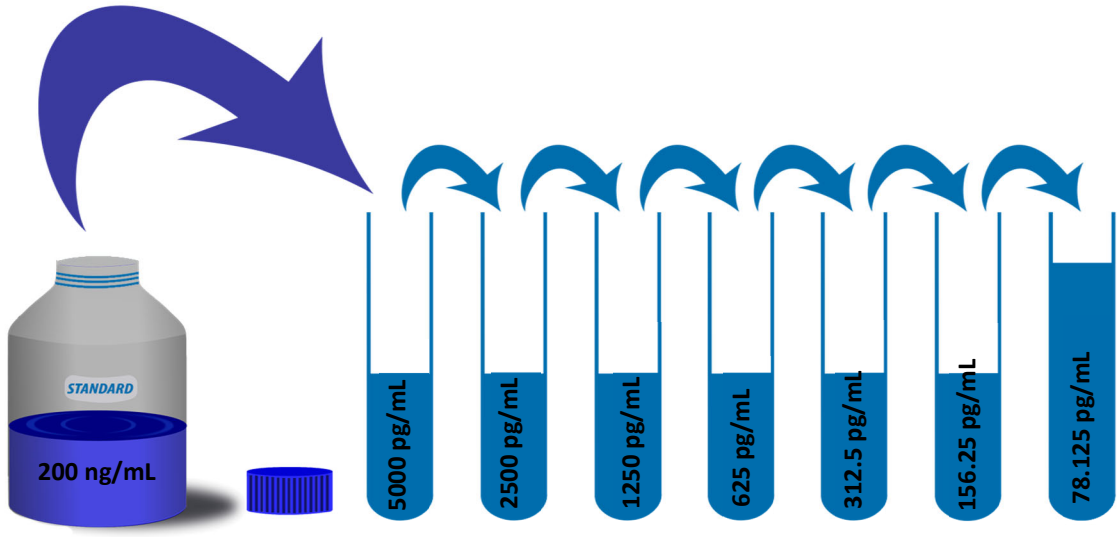
- For measuring T3 in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. The assay has been validated using RPMI-1640.

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 1:40 dilution of T3 Standard with Assay Buffer (mix 20 µL of standard with 780 µL of Assay Buffer), and label as the Standard No.7 (5000 pg/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.7	5000 pg/mL	20 µL T3 Standard + 780 µL Assay Buffer
Standard No.6	2500 pg/mL	250 µL Standard No.7 + 250 µL Assay Buffer
Standard No.5	1250 pg/mL	250 µL Standard No.6 + 250 µL Assay Buffer
Standard No.4	625 pg/mL	250 µL Standard No.5 + 250 µL Assay Buffer
Standard No.3	312.5 pg/mL	250 µL Standard No.4 + 250 µL Assay Buffer
Standard No.2	156.25 pg/mL	250 µL Standard No.3 + 250 µL Assay Buffer
Standard No.1	78.125 pg/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

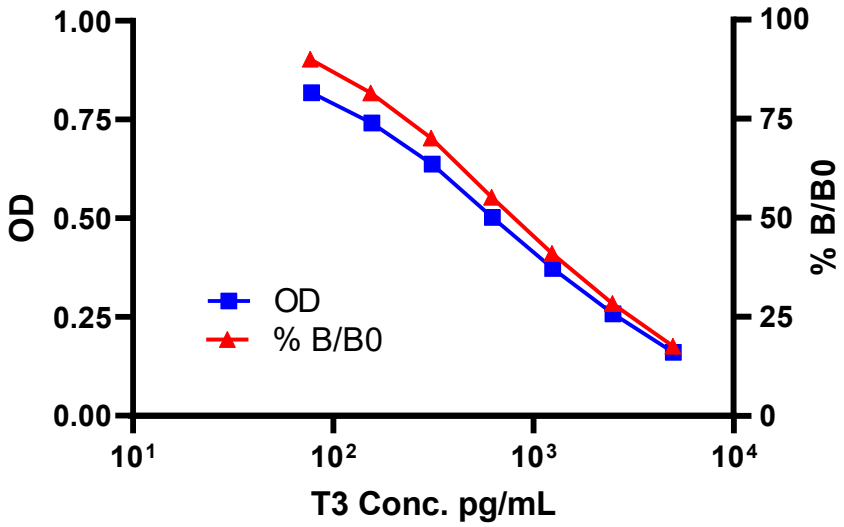
Assay Procedure

1. Pipette 100 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 100 µL of Assay Buffer into duplicate wells of the Zero standard.
3. Pipette 125 µL of Assay Buffer into duplicate wells of the nonspecific binding (NSB).
4. Add 25 µL of T3 Conjugate to each well, using a repeater pipette.
5. Add 25 µL of T3 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 2 hours at room temperature. If the plate is not shaken, signals will be approximately 20 % 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: 65.1 ng/mL of T3 is equivalent to 100 nM.



A typical standard curve of ZellX® T3 ELISA Kit kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® T3 ELISA Kit was determined as 46.6 pg/mL.

Sensitivity

The sensitivity of the ZellX® T3 ELISA Kit was determined as 37.4 pg/mL.

Precision

Intra-Assay Precision (Precision within an assay): 3 human samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 human samples were tested in duplicate on 20 different assays over multiple days.

Item	*%CV*
Intra assay	6.6, 6.7, 5.5
Inter assay	11.6, 14.7, 14

Cross Reactivity

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

Steroid	*Cross Reactivity (%)*
T3	100
Thyroxine (T4)	0.88
Reverse T3 (3,3',5'-Triiodo-L-thyronine)	< 0.1