Datasheet for ABIN2866595

Triiodothyronine T3 ELISA Kit

1 Image

Go to Product page

Overview

Quantity:	96 tests
Target:	Triiodothyronine T3 (T3)
Reactivity:	Various Species, Human, Mammalian
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The DetectX® Triiodothyronine (T) Enzyme Immunoassay kit is designed to quantitatively measure 3 T present in extracted serum and plasma, urine, extracted dried fecal samples, and tissue culture 3 media samples.
Brand:	DetectX®
Sample Type:	Cell Culture Supernatant, Fecal, Plasma (EDTA), Plasma (heparin), Serum, Urine
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Coated Clear 96 Well Plates A clear plastic microtiter plate(s) coated with donkey anti-sheep IgG. 1 or 5 Each Triiodothyronine (T) Standard, Triiodothyronine at 200 ng/mL in a special stabilizing solution. 70 µL or 350 µL DetectX® Triiodothyronine (T) Antibody 3 A sheep antibody specific for Triiodothyronine 3 mL or 13 mL DetectX® Triiodothyronine (T) Conjugate, Must be stored at -20°C. 3 A Triiodothyronine-peroxidase conjugate in a special stabilizing solution. 3 mL or 13 mL

Product Details

	Assay buffer Concentrate A 5X concentrate that must be diluted with deionized or distilled water. 28 mL or 55 mL Wash buffer Concentrate A 20X concentrate that must be diluted with deionized or distilled water. 30 mL or 125 mL TMB Substrate 11 mL or 55 mL Stop Solution A 1M solution of hydrochloric acid. 5 mL or 25 mL Plate Sealer 1 or 5 each
Material not included:	Distilled or deionized water. Repeater pipet with disposable tips capable of dispensing 25 µL, 50 µL and 100 µL. Colorimetric 96 well microplate reader capable of reading optical density at 450 nm. Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting.

Target Details

Target:	Triiodothyronine T3 (T3)
Alternative Name:	Triiodothyronine (T3) (T3 Products)
Target Type:	Amino Acid
Background:	Thyroid hormones regulate a number of developmental, metabolic, and neural activities throughout the body. The thyroid gland synthesizes 2 hormones: Thyroxine, which contains 4 atoms of iodine (T), 4 and triiodothyronine (T), which has 3 atoms of iodine. T production in the thyroid gland constitutes 3 3 approximately 20 % of the total T , the rest is generated by the conversion (deiodination) of T to T 3 4 3 in peripheral tissues. Circulating levels of T are much greater than T levels, but T is biologically the 4 3 3 most metabolically active hormone (3-4 times more potent than T) although its effect is briefer due 4 to its shorter half-life. Thyroid hormones circulate primarily bound to carrier proteins (eg, thyroid- binding globulin [TBG], prealbumin and albumin), whereas only a small fraction circulates unbound (free). The free form of T is the biologically active fraction. While both T and T are bound to TBG, T 3 3 4 3 is bound less firmly than T . Total T consists of both the bound and unbound fractions. 4 3 In hyperthyroidism, both T and T levels are usually elevated, but in a small subset of hyperthyroid 4 3 patients only T is elevated (T toxicosis). In hypothyroidism, T and T levels are decreased. T levels 3 3 4 3 3 are frequently low in sick or hospitalized euthyroid patients.

Application Details

Application Notes:	This assay has been validated for serum, EDTA and heparin plasma, urine and for tissue culture samples. It has also been validated for dried fecal extract samples. Samples containing visible particulate should be centrifuged prior to using. Moderate to severely hemolyzed samples should not be used in this kit. Triodothyronine is identical across all species and we expect this kit may measure Triodothyronine from sources other than human. The end user should evaluate recoveries of Triodothyronine in other samples being tested.
Plate:	Pre-coated
Protocol:	This kit measures total T in extracted serum and plasma and in extracted fecal samples. A T stock solution is provided to generate standard curves for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture sheep antibodies. A T -peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a sheep antibody to T to each well. After a two hour incubation the plate is washed and substrate is added. The substrate reacts with the bound T -peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450nm wavelength. The concentration of the T in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.
Reagent Preparation:	Allow the kit reagents to come to room temperature for 30 minutes. Assay buffer Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4 °C for 3 months. Wash buffer Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months. Standard Preparation Label test tubes as #1 through #7. Pipet 585 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #7. Carefully add 15 µL of the Triodothyronine stock solution to tube #1 and vortex completely. Take 300 µL of the Triodothyronine solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7.

	The concentration of Triodothyronine in tubes 1 through 7 will be 5,000, 2,500, 1,250, 625, 312.5, 156.25, and 78.125 pg/mL. Std 1 Std 2 Std 3 Std 4 Std 5 Std 6 Std 7 Assay buffer (µl) 585 300 300 300 300 300 300 Addition Stock Std 1 Std 2 Std 3 Std 4 Std 5 Std 6 Vol of Addition (µl) 15 300 300 300 300 300 300 Final Conc (pg/ mL) 5,000 2,500 1,250 625 312.5 156.25 78.125 Use all Standards within 2 hour of preparation.
Sample Preparation:	Serum and Plasma Samples: Serum and plasma samples need to be extracted. We would recommend the following protocol for serum and plasma. 1. Add ethyl acetate to serum or plasma samples at a 5:1 (v/v) solvent:sample ratio. 2. Mix solutions by vortexing for 2 minutes. Allow layers to separate for 5 minutes. 3. 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 steps 1-3 for maximum extraction efficiency, combining the solvent solutions. 4. 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. 5. Redissolve 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. Urine Samples Urine samples should be diluted at least 1:4 with the diluted Assay Buffer. For comparison to creatinine as a urine volume marker please see our NIST-calibrated Urinary Creatinine Detection kits, K002-H1 and K002-H5. dried Fecal Samples Dried fecal samples need to be extracted. The ethanol concentration in the final Assay Buffer dilution added to the well should be <5 % .
Assay Procedure:	ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine triiodothyronine concentrations. 1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4ºC. 2. Pipet 100 µL of samples or standards into wells in the plate. 3. Pipet 125 µL of Assay Buffer into the non-specific binding (NSB) wells. 4. Pipet 100 µL of Assay Buffer into wells to act as maximum binding wells (B0 or 0 ng/mL). 5. Add 25 µL of the DetectX® Triiodothyronine Conjugate to each well using a repeater pipet. 6. Add 25 µL of the DetectX® Triiodothyronine Antibody to each well, except the NSB wells, using a repeater pipet. 7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 20 % lower.

Application Details

	8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels. 9. Add 100 µL of the TMB Substrate to each well, using a repeater pipet. 10. Incubate the plate at room temperature for 30 minutes without shaking. 11. Add 50 µL of the Stop Solution to each well, using a repeater or a multichannel pipet. 12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm. 13. Use the plate reader's built-in 4PLC software capabilities to calculate Triiodothyronine concentration for each sample. NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.
Calculation of Results:	Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.
Restrictions:	For Research Use only

Handling

Precaution of Use:	As with all such products, 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. 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 other manufacturers Wash Buffers, containing sodium azide will inhibit color production from 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 as prepared. The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

Handling

Storage:	-20 °C,4 °C
Storage Comment:	The unopened kit must be stored at -20°C. Once opened the kit can be stored at 4°C up to the expiration date on the kit label, except for the Triiodothyronine (T) Standard and Triiodothyronine (T) Conjugate. These must be stored 3 3 at -20°C.

Images

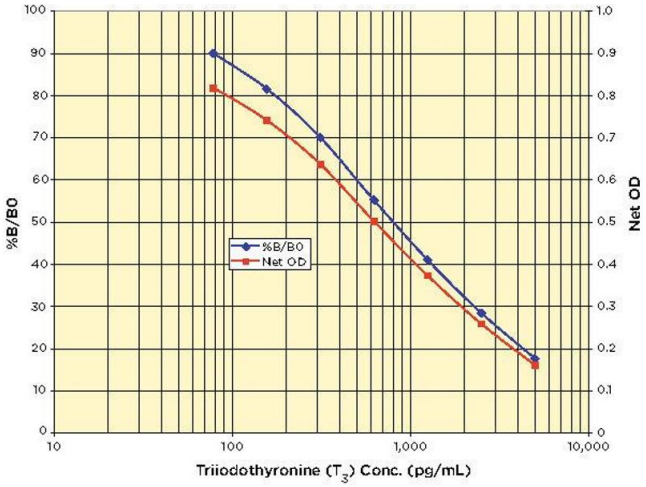

Image 1.