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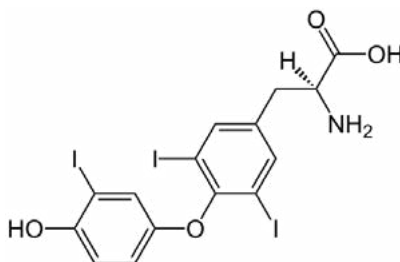
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BACKGROUND

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_3), which has 3 atoms of iodine. T_3 production in the thyroid gland constitutes approximately 20% of the total T_3 ; the rest is generated by the conversion (deiodination) of T_4 to T_3 in peripheral tissues. Circulating levels of T_4 are much greater than T_3 levels, but T_3 is biologically the most metabolically active hormone (3-4 times more potent than T_4) although its effect is briefer due 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_3 is the biologically active fraction. While both T_3 and T_4 are bound to TBG, T_3 is bound less firmly than T_4 . Total T_3 consists of both the bound and unbound fractions.

In hyperthyroidism, both T_4 and T_3 levels are usually elevated, but in a small subset of hyperthyroid patients only T_3 is elevated (T_3 toxicosis). In hypothyroidism, T_4 and T_3 levels are decreased. T_3 levels are frequently low in sick or hospitalized euthyroid patients.



SUPPLIED COMPONENTS

Coated Clear 96 Well Plates

A clear plastic microtiter plate(s) coated with donkey anti-sheep IgG.

Kit K056-H1 or -H5 1 or 5 Each

Catalog Number X061-1EA

Triiodothyronine (T₃) Standard

Triiodothyronine at 200 ng/mL in a special stabilizing solution.

Kit K056-H1 or -H5 70 µL or 350 µL

Must be stored at -20°C.

Catalog Number C216-70UL or -350UL

DetectX[®] Triiodothyronine (T₃) Antibody

A sheep antibody specific for Triiodothyronine

Kit K056-H1 or -H5 3 mL or 13 mL

Catalog Number C214-3ML or -13ML

DetectX[®] Triiodothyronine (T₃) Conjugate

A Triiodothyronine-peroxidase conjugate in a special stabilizing solution.

Kit K056-H1 or -H5 3 mL or 13 mL

Must be stored at -20°C.

Catalog Number C215-3ML or -13ML

Assay Buffer Concentrate

A 5X concentrate that must be diluted with deionized or distilled water.

Kit K056-H1 or -H5 28 mL or 55 mL

Catalog Number X065-28ML or -55ML

Wash Buffer Concentrate

A 20X concentrate that must be diluted with deionized or distilled water.

Kit K056-H1 or -H5 30 mL or 125 mL

Catalog Number X007-30ML or -125ML

TMB Substrate

Kit K056-H1 or -H5 11 mL or 55 mL

Catalog Number X019-11ML or -55ML

Stop Solution

A 1M solution of hydrochloric acid. **CAUSTIC.**

Kit K056-H1 or -H5 5 mL or 25 mL

Catalog Number X020-5ML or -25ML

Plate Sealer

Kit K056-H1 or -H5 1 or 5 Each

Catalog Number X002-1EA

STORAGE INSTRUCTIONS

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 (T₃) Standard and Triiodothyronine (T₃) Conjugate. These must be stored at -20°C.**

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Repeater pipet with disposable tips capable of dispensing 25, 50, 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. Contact your plate reader manufacturer for details.

PRECAUTIONS

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 on Page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.



SAMPLE TYPES

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. Triiodothyronine is identical across all species and we expect this kit may measure Triiodothyronine from sources other than human. **The end user should evaluate recoveries of Triiodothyronine in other samples being tested.**

SAMPLE PREPARATION

Triiodothyronine can be assayed in other sample types by using one of the extraction protocols available on our website at: www.arborassays.com/resources/#protocols

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%.

Tissue Culture Media

For measuring triiodothyronine 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. We have validated the assay using RPMI-1640.

Use all Samples within 2 Hours of preparation, or stored at ≤ -20°C until assaying.

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.

Or use the online tool from MyAssays to calculate the data:

www.myassays.com/arbor-assays-detectx-triiodothyronine-enzyme-immunoassay-kit.assay

TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	Triiodothyronine (T ₃) Conc. (pg/mL)
NSB	0.060	0.000	-	-
Standard 1	0.220	0.160	17.6	5,000
Standard 2	0.318	0.258	28.4	2,500
Standard 3	0.433	0.373	41.0%	1,250
Standard 4	0.562	0.502	55.2	625
Standard 5	0.697	0.637	70.1	312.5
Standard 6	0.801	0.741	81.5	156.25
Standard 7	0.878	0.818	90.0	78.125
B0	0.969	0.909	100	0
Sample 1	0.555	0.495	54.4	662.9
Sample 2	0.449	0.389	42.8	1,147.7

Always run your own standard curve for calculation of results. Do not use this data. Conversion Factor: 65.1 ng/mL of Triiodothyronine (T₃) is equivalent to 100 nM.



Intra Assay Precision

Three human samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated triiodothyronine concentrations were:

Sample	Triiodothyronine Conc. (pg/mL)	%CV
1	629.5	6.6
2	1,169.3	5.5
3	541.6	6.7

Inter Assay Precision

Three human samples were diluted with Assay Buffer and run in duplicates in twenty assays run over multiple days by four operators. The mean and precision of the calculated thyroxine concentrations were:

Sample	Triiodothyronine Conc. (pg/mL)	%CV
1	682.4	11.6
2	1,193.8	14.0
3	587.7	14.7

SAMPLE VALUES

Twenty-one random mammalian serum samples were tested in the assay. Extracted sample values ranged from 246 to 1,105 pg/mL with a mean of 610 pg/mL. Sixteen random mammalian extracted plasma samples were tested in the assay. Sample values ranged from 189 to 810 pg/mL with a mean of 446.6 pg/mL.

Sixteen random urine samples were tested in the assay. Adjusted values for the samples were 187.2 and 2,337 pg/mL with a mean of 1,010.9 pg/mL.

Eleven dried fecal samples from Tigers, Muntjac, Lion, Kudu, Giraffe, Fennec Fox, and Clouded Leopards were run in the assay. Values ranged from 13.0 to 96.0 pg/mg dry fecal weight with a mean of 47.3 pg/mg dry fecal weight.

CROSS REACTIVITY

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

Steroid	Cross Reactivity (%)
Triiodothyronine (T ₃)	100%
Thyroxine (T ₄)	0.88%
Reverse T3 (3,3',5'-Triiodo-L-thyronine)	<0.1%

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.

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