ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

Triiodothyronine (T_3)
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K056-H1
5 Plate Kit Catalog Number K056-H5

Species Independent

Sample Types Validated:

**Serum, EDTA and Heparin Plasma,
Urine, TCM and Fecal Extracts**

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

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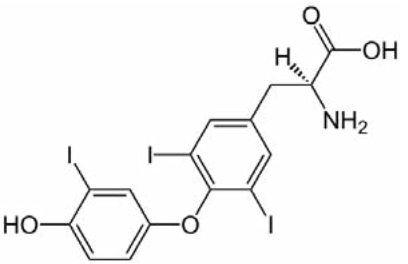
BACKGROUND

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.



ASSAY PRINCIPLE

The DetectX® Triiodothyronine (T_3) Enzyme Immunoassay kit is designed to quantitatively measure T_3 present in extracted serum and plasma, urine, extracted dried fecal samples, and tissue culture media samples. Please read the complete kit insert before performing this assay. This kit measures total T_3 in extracted serum and plasma and in extracted fecal samples.

A T_3 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_3-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_3 to each well. After a two hour incubation the plate is washed and substrate is added. The substrate reacts with the bound T_3-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 450 nm wavelength. The concentration of the T_3 in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
Urinary Creatinine Detection Kits (2 or 10 Plates)	K002-H1/H5
Throxine (T4) EIA Kits	K050-H1/H5
Cortisol Enzyme Immunoassay Kits (Strip Wells)	K003-H1/H5
Cortisol Enzyme Immunoassay Kits (Whole Plate)	K003-H1W/H5W
Glucose Colorimetric Detection Kit (2 Plate)	K039-H1
Glucose Fluorescent Detection Kit (2 Plate)	K039-F1

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 (T_3) Standard
Must be stored at -20°C.

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

Kit K056-H1 **or** -H5 70 µL **or** 350 µL — Catalog Number C216-70UL **or** -350UL

DetectX® Triiodothyronine (T_3) 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 (T_3) Conjugate
Must be stored at -20°C.

A Triiodothyronine-peroxidase conjugate in a special stabilizing solution.

Kit K056-H1 **or** -H5 3 mL **or** 13 mL — 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 (T_3) Standard and Triiodothyronine (T_3) 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. 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.**

SAMPLE PREPARATION

Triodothyronine 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 triodothyronine 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.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes.

Assay Buffer

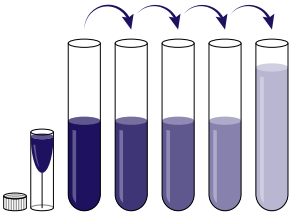
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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.

ASSAY PROTOCOL

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 4˚C.
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 the maximum binding (B0 or Zero standard) wells.
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. We recommend shaking at around 700–900 rpm. If the plate is not shaken, signals bound will be approximately 20% lower.
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.

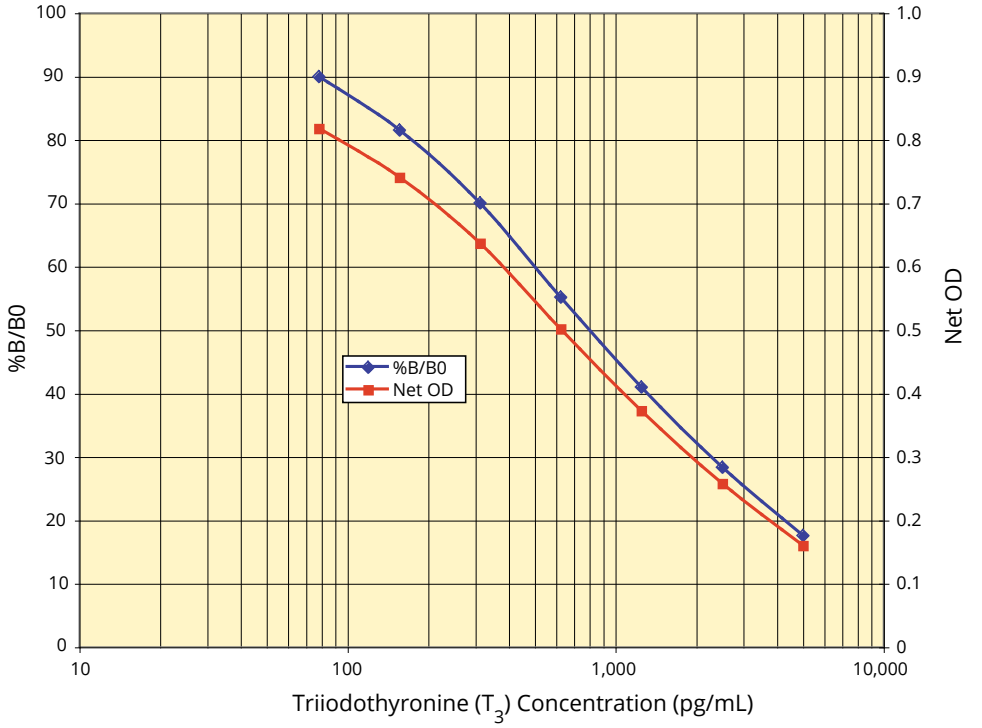
Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-detectx-triiodothyronine-enzyme-immunoassay-kit.assay

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 (T_3) is equivalent to 100 nM.

Typical Standard Curve



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #7. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.

Sensitivity was determined as 37.4 pg/mL.

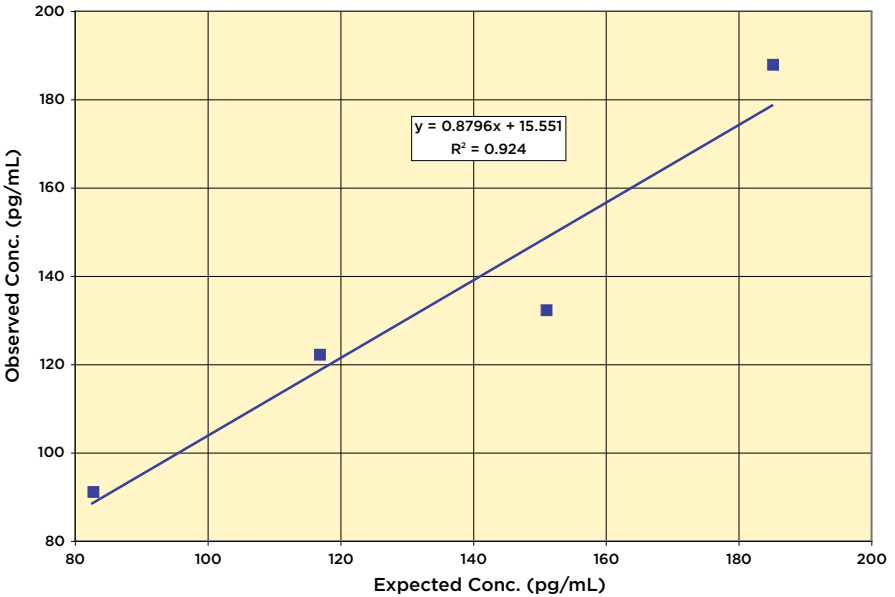
The Limit of Detection was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample.

Limit of Detection was determined as 46.6 pg/mL

Linearity

Linearity was determined using urine samples, by taking samples with a high known triiodothyronine concentration and a lower triiodothyronine concentration and mixing them in the ratios given below. The measured triiodothyronine concentrations were compared to the expected values based on the ratios used.

High Sample	Low sample	Expected Comc. (pg/mL)	Observed Comc. (pg/mL)	% Recovery
80%	20%	185.2	187.9	101.5
60%	40%	151.0	132.3	87.6
40%	60%	116.9	122.2	104.6
20%	80%	82.8	91.1	110.1
				100.9%



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 Leapords 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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ASSAYS™
FOR SERIOUS SCIENCE

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