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BIOGENIC AMINES & NEUROSCIENCE | ENDOCRINOLOGY | FOOD SAFETY
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Please use only the valid version of the Instructions for Use provided with the kit

Instructions for use

FT3 ELISA 2nd Generation

REF TF E-2100R

2°C – 8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

INTENDED USE

For the direct quantitative determination of Free Triiodothyronine by an enzyme immunoassay in human serum. For *research* use only.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of fT3 in the sample. A set of standards is used to plot a standard curve from which the amount of fT3 in samples and controls can be directly read.

The labelled T3 (conjugate) employed in this assay system has shown no substantial binding properties towards thyroxine-binding globulin (TBG) or human serum albumin (HSA). The binding sites on the microplates are designed to be of a low binding-capacity in order not to disturb the equilibrium between T3 and its carrying proteins. The assay is carried out under normal physiological conditions of pH, temperature and ionic strength.

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A standard curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of fT3 in human serum. The kit is not calibrated for the determination of fT3 in other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric orimproperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Samples reading higher than 40 pg/ml should be reported as such and should not be diluted. Dilution will alter the existing equilibrium and may lead to false results.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

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CHEMICAL HAZARDS
Avoid contact with reagents containing TMB, hydrogenperoxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE
Approximately 0.1 ml of serum is required per duplicate determination. Collect 4 – 5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4 °C for up to 24 hours or at -10 °C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT
This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED
1. Precision pipette to dispense 25, 50, 100, 150 and 300 µl
2. Disposable pipette tips
3. Distilled or deionized water
4. A 37 °C incubator
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 11)

REAGENTS PROVIDED

1. AA E-0030 WASH-CONC 10x **Wash Buffer Concentrate** – Requires Preparation **X10**

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume:	50 ml/bottle
Storage:	Refrigerate at 2 – 8 °C
Stability:	12 months or as indicated on label.
Preparation:	Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

2. AA E-0055 SUBSTRATE **TMB Substrate** – Ready To Use.

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume:	16 ml/bottle
Storage:	Refrigerate at 2 – 8 °C
Stability:	12 months or as indicated on label.

3. AA E-0080 STOP-SOLN **Stopping Solution** – Ready To Use.

Contents:	One vial containing 1M sulfuric acid.
Volume:	6 ml/bottle
Storage:	Refrigerate at 2 – 8 °C
Stability:	12 months or as indicated on label.
Hazards identification:	

H315 Causes skin irritation.
H319 Causes serious eye irritation.

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4. Standards and **Controls** – Ready To Use.

Listed below are approximate concentrations, please refer to bottle labels for exact concentrations:

Cat. no.	Symbol	Standard	Concentration	Volume/vial
TF E-2101	STANDARD A	**Standard A**	0 pg/ml	0.5 ml
TF E-2102	STANDARD B	**Standard B**	2 pg/ml	0.5 ml
TF E-2103	STANDARD C	**Standard C**	4 pg/ml	0.5 ml
TF E-2104	STANDARD D	**Standard D**	8 pg/ml	0.5 ml
TF E-2105	STANDARD E	**Standard E**	16 pg/ml	0.5 ml
TF E-2106	STANDARD F	**Standard F**	40 pg/ml	0.5 ml
TF E-2151	CONTROL 1	**Control 1**	Refer to vial labels for expected value and acceptable range!	0.5 ml
TF E-2152	CONTROL 2	**Control 2**		0.5 ml

Contents: fT3 in a human serum-based matrix with a non-mercury preservative. Prepared by spiking serum with a defined quantity of T3.

Storage: Refrigerate at 2 – 8 °C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards and controls should be used within 14 days or aliquoted and stored frozen.
Avoid multiple freezing and thawing cycles.

5. TF E-2113 ASSAY-BUFF **Assay Buffer** – Ready To Use.

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.

Volume: 15 ml/bottle

Storage: Refrigerate at 2 – 8 °C

Stability: 12 months or as indicated on label.

6. TF E-2131 96 **Rabbit Anti-fT3 Antibody-Coated Break-Apart Well Microplate** – Ready To Use.

Contents: One 96 well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.

Storage: Refrigerate at 2 – 8 °C

Stability: 12 months or as indicated on label.

7. TF E-2140 CONJUGATE-CONC 50x **fT3-Horseradish Peroxidase (HRP) Conjugate Concentrate –** Requires Preparation **X50**

Contents: fT3-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 300 µl/vial

Storage: Refrigerate at 2 – 8 °C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µl of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µl of HRP in 12 ml of assay buffer. Discard any that is left over.

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ASSAY PROCEDURE

Specimen Pretreatment: **None**.

All reagents must reach room temperature before use. Standards, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1.	**Prepare** working solutions of the **fT3-HRP conjugate** and **wash buffer**.
2.	Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3.	Pipette **25 µl** of each **standard, control and specimen sample** into correspondingly labelled wells in duplicate.
4.	Pipette **100 µl** of the **conjugate working solution** into each well. *(We recommend using a multichannel pipette.)*
5.	Gently **shake** the plate for **10 seconds**.
6.	**Incubate** the plate at **37 °C for 1 hour**.
7.	Wash the wells 3 times with **300 µl** of **diluted wash buffer** per well and tap the plate firmly against absorbent paper to ensure that it is dry. *(The use of a washer is recommended.)*
8.	Pipette **150 µl** of **TMB substrate** into each well at timed intervals.
9.	**Incubate** the plate at **37°C** for **10–15 minutes.** *(or until standard A attains dark blue colour for desired OD).*
10.	Pipette **50 µl** of **stopping solution** into each well at the same timed intervals as in step 8.
11.	**Read** the plate on a microplate reader at **450 nm** within 20 minutes after addition of the stopping solution.
⚠	*If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of donor/control samples.*

CALCULATIONS

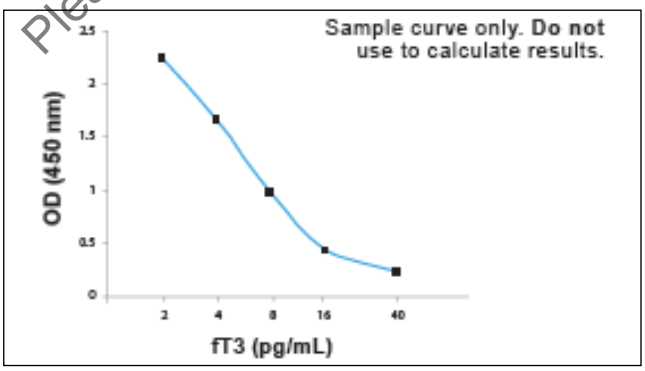
1. Calculate the mean optical density of each standard duplicate.
2. Draw a standard curve on semi-log paper with the mean optical densities on the Y-axis and the standard concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the standard curve.

TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Standard	OD 1	OD 2	Mean OD	Value (pg/ml)
A	2.798	2.734	2.764	0
B	2.284	2.216	2.250	2
C	1.697	1.638	1.668	4
D	1.002	0.956	0.979	8
E	0.452	0.437	0.444	16
F	0.247	0.238	0.242	40
Unknown	1.460	1.439	1.450	4.8

TYPICAL STANDARD CURVE



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PERFORMANCE CHARACTERISTICS

SENSITIVITY
The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of standard A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the Direct fT3 ELISA kit is **0.3 pg/ml**.

SPECIFICITY (CROSS REACTIVITY)
The following compounds were tested for cross-reactivity with the Direct fT3 ELISA kit with T3 cross-reacting at 100%:

Compound	% Cross Reactivity
L-Triiodothyronine	100
D-Triiodothyronine	34
Triiodothyropropionic acid	20
Diiodo-D-thyronine	0.5
D-Thyroxine	0.3
L-Thyroxine	0.9

The following compounds were tested but cross-reacted at less than 0.1%: Diiodotyrosine, Iodotyrosine, Phenytoin, Sodium Salicylate and r-Triiodothyronine.

INTRA-ASSAY PRECISION
Three samples were assayed ten times each on the same standard curve. The results (in pg/ml) are tabulated below:

Sample	Mean	SD	CV%
1	5.182	0.501	9.7
2	8.560	0.598	7.0
3	48.200	1.686	3.5

INTER-ASSAY PRECISION
Three samples were assayed ten times over a period of four weeks. The results (in pg/ml) are tabulated below:

Sample	Mean	SD	CV%
1	3.306	0.284	8.6
2	5.154	0.402	7.8
3	8.698	0.713	8.2

EXPECTED NORMAL VALUES
Each laboratory should collect data and establish their own range of expected normal values. The following reference range (pg/ml) was established with 44 apparently healthy adults:

Group	N	Mean	Central 95% Range
Euthyroid Adults	44	3.7	2.2 – 5.3

EFFECT OF THYROXINE BINDING GLOBULIN (TBG)
The purpose of this study was to investigate a possible interference caused by the binding of TBG to the fT3-HRP conjugate. The standard A was spiked with purified TBG and assayed.
The results are tabulated below:

TBG (µg/ml added)	OD	% B/B_0
0	1.255	100
12.5	1.229	98
25	1.170	93
50	1.137	91
100	1.168	93
200	1.174	94
400	1.118	89

The results show no binding of labelled T3 to TBG even at higher than normal levels. In conclusion, results showed that there was no significant influence by TBG in the Direct Free T3 Direct ELISA Kit.

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EFFECT OF HUMAN SERUM ALBUMIN (HSA)

The purpose of this study was to investigate a possible interference of HSA on the assay procedure. The Standard A was spiked with purified HSA and assayed.
The results are tabulated below:

HSA (mg/ml added)	OD	% B/B_0
0	1.255	100
3.125	1.228	98
6.25	1.331	100
12.5	1.245	99
25	1.197	95
50	1.217	97
100	1.063	85

The results show no significant binding of labelled T3 to HSA even at higher than normal levels.

EFFECT OF NON-ESTERIFIED FATTY ACIDS (NEFA)

The purpose of this study was to investigate a possible interference of NEFA on the assay procedure. Two samples were spiked with oleic acid and assayed.
The results are tabulated below:

NEFA (mmol/l added)	Sample 1 (pg/ml)	Sample 2 (pg/ml)
0	4.4	8.7
0.5	4.6	7.5
3.5	4.6	8.3
25	4.6	10.9

The results show that NEFA may increase the free T3 values, only at higher than normal concentrations.

EFFECT OF LIPEMIA

The purpose of this study was to investigate a possible interference of lipemic samples on the assay procedure. Two samples were spiked with triglycerides and assayed.
The results are tabulated below:

Triglycerides (mg/dl added)	Sample 1 (pg/ml)	Sample 2 (pg/ml)
0	4.4	8.7
50	5.5	9.9
75	5.9	10.8

Results show that lipemic samples may increase the free T3 values. Therefore, lipemic samples should not be used in this assay.

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CHANGE HISTORY

Previous Version:	6.0a-r	**New Version:**	6.0b-r
Changes:	***REAGENTS PROVIDED*** Hazard labelling for component AA E-0080 updated		

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number		Distributor
	Date of manufacture			RUO	For research use only!