DRG

DRG® T4-Free (CLA-4659)

CE

REVISED 23 AUG. 2010 RM (VERS. 1.1) **RUO IN THE USA**

1 INTENDED USE I

For determination of Free Thyroxine n human serum by chemiluminescence immunoassay (CLIA).

In the United States, this kit is intended for Research Use Only.

2 PRINCIPLE OF THE TEST

The principle of the following chemiluminescence immunoassay (CLIA) test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, control and donor samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the luminescence substrate solution is added. The relative luminescence units (RLUs) are measured on a microtiter plate luminometer. The RLU values are inversely proportional to the concentration of fT4 in the sample. A set of calibrators are used to plot a standard curve from which the amount of fT4 in donor samples and controls can be directly read.

The labeled T4 (conjugate) employed in this assay system has shown no binding properties towards thyroxine-binding globulin (TBG) and human serum albumin (HSA). The binding sites on the microwell plates are designed to be of a low binding-capacity in order not to disturb the equilibrium between T4 and its carrying proteins. The assay is carried out under normal physiological conditions of pH, temperature and ionic strength.

3 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 calibrator curve must be established for every run.
7. The kit control 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 control do
9. The luminescence substrate solutions (A and B) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
10. When dispensing the substrate, do not use pipettes in which these liquids will come into contact with any metal parts.
11. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
12. 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.
13. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

4 LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination free T4 in human serum. The kit is not calibrated for the determination of free T4 in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly 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 100 pg/ml should be reported as such and should not be diluted. Dilution will alter the existing equilibrium and may lead to false results.
5. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any donors who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

5 SAFETY CAUTIONS AND WARNINGS

Human serum that may be used in the preparation of the standards and control 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. However no test method 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.

CHEMICAL HAZARDS

Avoid direct contact with reagents. In case of contact, wash with plenty of water.

6 SPECIMEN COLLECTION AND STORAGE

Approximately 0.2 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.

7 SPECIMEN PRETREATMENT

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

8 REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25, 100, 150 and 300 µl
2. Disposable pipette tips
3. Distilled or deionized water
4. A 37 °C incubator
5. Plastic wrap or microtiter plate cover.

6. Microwell plate luminometer

9 REAGENTS PROVIDED AND PREPARATION

1. Mouse Anti-fT4 Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use.

Contents: One 96 well (12x8) monoclonal antibody-coated microwell plate in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

2. fT4-Horseradish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.

Contents: fT4-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 12ml of assay buffer. Discard any that is left over.

3. fT4 Calibrators - Ready To Use.

Contents: Five vials containing fT4 in a human serum-based matrix with a non-mercury preservative. Prepared by spiking serum with an exact quantity of T4, then free T4 concentrations were determined.

*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator A	0 pg/ml	0.5 ml
Calibrator B	2 pg/ml	0.5 ml
Calibrator C	8 pg/ml	0.5 ml
Calibrator D	40 pg/ml	0.5 ml
Calibrator E	120 pg/ml	0.5 ml

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing an0d thawing cycles.

4. Control - Ready To Use.

Contents: One vial containing fT4 in a human serum-based matrix with a non-mercury preservative. Prepared by spiking serum with a defined quantity of T4. Refer to vial label for expected value and acceptable range.

Volume: 0.5 ml/vial

Storage: Refrigerate at 2-8 °C

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

5. Wash Buffer Concentrate - Reburies Preparation.

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.

6. Assay Buffer - Ready to Use.

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

Volume: 15 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

7. Chemiluminescence Substrate Reagent A - Requires Preparation.

Contents: One bottle containing luminol enhancer.

Volume: 1.2 ml/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: See below.

8. Chemiluminescence Substrate Reagent B - Requires Preparation.

Contents: One vial containing peroxide solution.

Volume: 1.2 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: See below.

9. Chemiluminescence Substrate Reagent C - Requires Preparation.
Contents: One vial containing buffer with a non-mercury preservative.
Volume: 15 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: See below.

Preparation of Working Substrate Solution:
Mix **1 part** of the chemiluminescence **substrate reagent A** with **1 part of reagent B** and dilute this mixture **1:5 with reagent C**. This gives the ready to use substrate solution. Prepare fresh for each use.
If the whole plate is to be used prepare working substrate solution as follows:
Combine 1 ml of reagent A with 1 ml of reagent B. To the 2 ml of this mixture add 10 ml of reagent C.
Total volume=12 ml of working substrate solution.
Stability: Working substrate solution is stable for 24 hours at room temperature.

10 ASSAY PROCEDURE

Specimen Pretreatment: None.

Important Notes:
1. All reagents must reach room temperature before use.
2. Once the procedure has been started, all steps should be completed without interruption to ensure equal elapsed time for each pipetting step.
3. The washing procedure influences the precision markedly; it is essential to ensure the washing is effective and thorough.

Procedure:
1. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
2. Pipette 25 µl of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
3. Pipette 100 µl of the conjugate working solution into each well
(We recommend using a multichannel pipette).
4. Cover plate and incubate for 60 minutes in a 37 °C incubator.
5. Wash the wells 5 times, each time with 300 µl of diluted wash buffer per well and on the last washing tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).

6. Pipette 100 µl of chemiluminescence working substrate solution into each well (We recommend using a multichannel pipette).
7. Incubate without shaking for 15 minutes at room temperature.
8. Measure the RLUs in each well on a microplate luminometer within 20 minutes after addition of the substrate.

11 CALCULATIONS

1. Calculate the mean RLU of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean RLUs on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean RLU of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. 5 Samples reading higher than the highest calibrator should be reported as such and should not be diluted. Dilution will alter the existing equilibrium and may lead to false results.

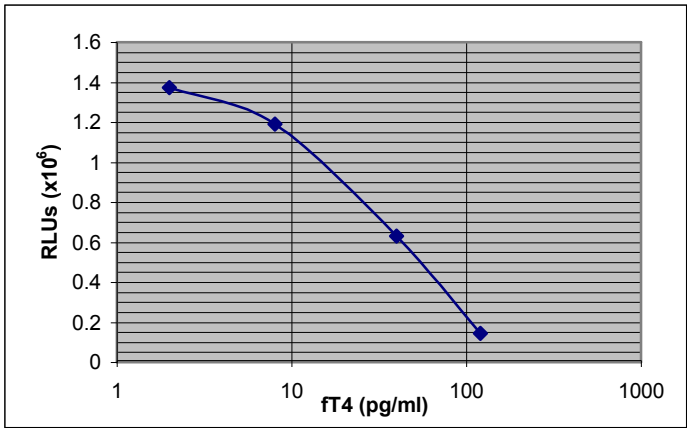
TYPICAL TABULATED DATA**

Calibrator	RLU 1 x 103	RLU 2 x 103	Mean RLU x 103	RLU/RLUMAX (%)
A, 0 pg/ml	1503	1480	1492	100
B, 2 pg/ml	1364	1381	1372	92
C, 8 pg/ml	1172	1209	1191	80
D, 40 pg/ml	625	635	630	42
E, 120 pg/ml	143	144	144	9.6

**- It is recommended to use the RLU/RLU_{MAX} values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show quite different RLU values, however, the RLU/RLU_{MAX} values remain consistent.

TYPICAL CALIBRATOR CURVE

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



11.1 EFFECT OF BILIRUBIN

Bilirubin was added to a donor sample at concentrations of 50 and 100 µg/ml and assayed with the DRG fT4 CLIA kit. Results are tabulated below:

Sample	fT4 (pg/ml)
Unspiked	8.78
+ 50 µg/ml bilirubin	10.68
+100 µg/ml bilirubin	9.72

No significant effect was observed at these concentrations.

11.2 EFFECT OF HUMAN SERUM ALBUMIN (HSA)

Purified human serum albumin (HSA) was added to a donor sample at concentrations of 10, 20 and 40 mg/ml. Samples were assayed with the DRG fT4 CLIA kit. Results are tabulated below:

Sample	fT4 (pg/ml)
Unspiked	8.78
+10 mg/ml	8.81
+20 mg/ml	9.46
+40 mg/ml	9.90

No binding of labelled fT4 to HSA was found at these concentrations.

11.3 EFFECT OF THYROXINE-BINDING GLOBULIN (TBG)

The zero calibrator was spiked precisely with purified TBG at concentrations ranging from 25-200 µg/ml and assayed with the DRG fT4 CLIA kit. Results are tabulated below:

Sample	TBG Added (µg/ml)	RLU (x106)
1	0	1.294
2	25	1.390
3	50	1.472
4	100	1.490
5	200	1.542

No significant binding of labelled fT4 to TBG was found at these concentrations.

11.4 EFFECT OF NON-ESTERIFIED FATTY ACIDS

Oleic acid was added to a donor sample at concentrations of 0.5, 5 and 20 mmol/L and assayed with the DRG fT4 CLIA kit. Results are tabulated below:

Sample	fT4 (pg/ml)
Unspiked	24.83
+0.5 mmol/L	20.53
+5 mmol/L	26.06
+20 mmol/L	83.64

At high concentrations of oleic acid, the free T4 level was significantly increased. This is due to the well-known effect that non-esterified fatty acids can dissociate T4 from its carrier proteins.

12 REFERENCES

1. Ingbar,S.H.et at., J.Clin. Invest, 44:1679, 1965
2. Robins, J., Metabolism, 22(8):1021, 1973
3. Schall, R.F. J., Clin.Chem., 24(10):1801, 1978
4. Selenkow,H.A. and Robin, N.I., J.Maine Med. Assoc.,61:199, 1970
5. Oppenheimer, J.H. et. al. J. Clin. Invest., 42:1769, 1963
6. Young, D.S., et. al., Clin. Chem., 21:3640, 1975
7. Sterling, K., and Hegedus, A.J. Clin. Invest., 41:1031, 1962
8. Cavalieri, R.R., et. al., Clin. Res., 15:124, 1967
9. Comoglio, S. and Celada, F.J., Immunol. Meth., 10:161-170, 1976
10. McComb, R. B., Bowers, G.N., Posen, S., Alkaline Phosphatase, 1st Ed., Chap. 9, pg. 525-704, Plenum Press, New York, 1979