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CALBIOTECH
A life science company

Neonatal Thyroxine (T4) ELISA

Catalog No.: T4381N (96 Tests)

INTENDED USE

The Calbiotech, Inc., Neonatal Thyroxine ELISA kit is intended for the quantitative determination of total T4in Human whole blood.

PRINCIPLE OF THE TEST

The Calbiotech Inc., Neonatal Thyroxine ELISA is a competitive solid phase ELISA that uses a whole blood spot sample dried on WHATMAN type 903 filter paper. In the assay, streptavidin coated wells are incubated with dry blood spots (DBS), 50µl T4-HRP conjugate, and 50µl mouse anti-T4 MAb. T4-HRP conjugate competes with T4 molecules in the standards and samples for a fixed number of binding sites of the specific anti-T4 antibody. Thus, the amount of T4-peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of thyroxine in the specimen increases. Unbound T4 is then removed by the washing steps. TMB Substrate is added, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is spectrophotometrically measured at 450nm. A standard curve is prepared relating color intensity to the concentration of Thyroxine.

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	12x8x1
2. T4 Standards, 6 Dried Blood Spots	6 DBS
3. T4 Controls, 2 Dried Blood Spot per level, per card	6 DBS
4. Antibody anti-T4Reagent: 1 bottle (ready to use)	7 mL
5. T4 Enzyme Conjugate, 7X:1 vial	1.2 mL
6. N-T4Assay Diluent: 1 bottle	7 mL
7. TMB Substrate: 1 bottle (ready to use)	12 mL
8. Stop Solution: 1 bottle (ready to use)	12 mL
9. Wash Concentrate 20X: 1 bottle	25 mL

MATERIALS NOT PROVIDED

1. 1/8" inch hole punch
2. Microplate shaker
3. Distilled or deionized water
4. Precision pipettes
5. Disposable pipette tips
6. ELISA reader capable of reading absorbance at 450 nm
7. Absorbance paper or paper towel
8. Graph paper

STORAGE ANDSTABILITY

1. Store the kit at 2-8oC.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

SPECIMEN COLLECTION AND HANDLING

Follow the guidelines in the NCCLS publication LA4T7 for collecting blood samples in the neonatal screening program, copies of which can be obtained from: NCCLS, 771 E. Lancaster Ave, Villanova, PA 19085. Use WHATMAN type 903 filter paper. For samples screening for CAH, collect samples 3 to 5 days after birth. Use disposable lancets with tips less than 2.5 mm to prick the medial or lateral sides of the bottom of the heel. Allow a drop of blood to form with sufficient volume to fill a 5/8-inch diameter spot on filter paper. Gently touch the drop of blood with the filter paper. **DO NOT PRESS AGAINST THE SKIN. DO NOT TOUCH SPOTTED AREA**. Suspend spotted papers horizontally and allow drying at room temperature for a minimum of 3 hours. Avoid spots touching other surfaces and keep away from direct light. The samples should be transported to the laboratory within 24 hours after collection in appropriate storage container. The laboratory should store the specimens at 2-8 °C protected from moisture and direct light. The dried blood spots are stable for at least 3 weeks at 2-8 °C protected from light and moisture. Reject samples with the following conditions:

1. Specimens not collected on WHATMAN type 903 filter paper.
2. Blood spots not completely saturated on both sides.
3. Blood spots with appearance of caking or clotting.
4. Blood spots with appearance of moisture

REAGENT PREPARATION

1. **Working T4 Enzyme Conjugate**: Prepare 1X working dilution at 1:7 with assay diluents as needed, e.g. 0.3ml of the stock conjugate plus 1.8ml of assay diluent for 40 wells. The diluted conjugate has to be used on the same day.
2. **Wash Buffer**: Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475ml of distilled water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Punch out 1/8" blood spot out of each standard and samples into the assigned wells. (NOTE: Do not punch blood spots from areas that are printed or that are near the edge of the blood spot).
3. Add 50µl of working dilution T4 enzyme conjugate to all the wells.
4. Add 50 µl of Antibody anti-T4 Reagent to all wells.
5. Shake the microplate gently for 20-30 seconds to mix. Make sure that all blood spots are fully submerged in the liquid and not stuck to the walls of the wells.
6. Cover the microplate and put it on a microplate shaker at 800rpm to 1000rpmfor 120 min, RT
7. Remove the contents of the wells by decantation or aspiration. Make sure all the blood dots are removed at this point. Rinse the wells 3 times with 1X wash buffer. Strike the wells sharply to remove residual wash buffer droplets.
8. Add 100µl of TMB substrate to each well.
9. Cover the microplate and incubate for 15 minutes, at room temperature with shaking at 800-1000 rpm.
10. Add 50µl of stop solution to each well and gently mix until a uniform color, in each well, is obtained.
11. Read the absorbance in each well at 450nm within 15 minutes after adding the stop solution.

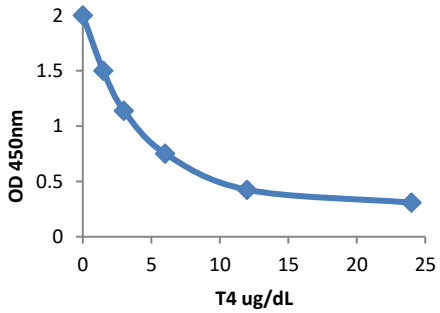
CALCULATION OF RESULTS

A standard curve is constructed as follows:

1. Calculate the average absorbance values for each set of standards and patient samples
2. To construct the standard curve, plot the mean absorbance of each T4 standard (vertical axis) against its concentration in ug/dL (horizontal axis)
3. Draw the best-fit curve through the plotted points.
4. Read the absorbance for each unknown sample from the curve to determine the corresponding concentration of total T4.

Example of a Typical Standard Curve for Neonatal Thyroxine

	OD450nm	Conc. (ug/dL)
Std 1	2.0	0
Std 2	1.50	1.5
Std 3	1.14	3
Std 4	0.753	6
Std 5	0.425	12
Std 6	0.308	24



EXPECTED VALUES

We recommend each laboratory to establish its own normal ranges, for the population it serves. Until then, literature values may be used as guidelines, where healthy neonates range is 8-23 ug/dL.

REPORTABLE RANGE: Analytical Range = 1.5- 24 ug/dL Samples that fall within the calibration curve should be reported as such.