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Cat #: TS343N (96 Tests)


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CALBIOTECH
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# NEONATAL TSH ELISA

Catalog No.: TS343N (96 Tests)

## INTENDED USE

The Calbiotech, Inc. Neonatal Thyroid Stimulating Hormone (TSH) ELISA kit is intended for the quantitative determination of TSH in Human whole blood. For Research Use Only. Not for use in Diagnostic Procedures.

## SUMMARY AND EXPLANATION

Determination of hypothyroidism within the first few days of birth has been recognized as the single most important diagnostic tests in neonates by the American Thyroid Association. The need for its early detection and treatment hast resulted in the establishment of screening centers by federal and state health departments. A program of early screening of neonates for congenital hypothyroidism was started in Quebec, Canada in the early seventies. They used dry blood spots on filter paper as the sampling device. Very soon the program was followed by other major public health institutions in Canada and the US. By 1978 almost one million infants had been screened and an incidence rate of congenital hypothyroidism was established to be approximately 1 in 7000 births.
Congenital hypothyroidism is probably the single most common preventable cause of mental retardation. Diagnosis and treatment of congenital hypothyroidism within the first 1-2 months after birth appears to be necessary in order to prevent severe mental retardation.

## PRINCIPLE OF THE TEST

The Calbiotech Neonatal Thyroid Stimulating Hormone (TSH)) is a sandwich ELISA in blood spot dried on WHATMAN type 903 filter paper. In the assay, streptavidin coated wells are incubated with blood spots and biotin-conjugated monoclonal antibody anti TSH to form a streptavidin-biotin mAb-TSH complex. After washing steps, other HRP-labeled mAb anti TSH is added to recognize the previous complex and complete the sandwich. Unbound HRP-labeled mAb 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 TSH.

| MATERIALS PROVIDED | 96 Tests |
|---|---|
| 1. Microwells coated with Streptavidin | 12x8x1 |
| 2. N-TSH Standards, 6 Dried Blood Spots | 6 DBS |
| 3. N-TSH Controls, 2 Dried Blood Spot per level, per card | 6 DBS |
| 4. N-TSH Biotin Reagent: 1 bottle (ready to use) | 12 mL |
| 5. N-TSH Enzyme Reagent: 1 bottle (ready to use) | 12 mL |
| 6. TMB Substrate: 1 bottle (ready to use) | 12 mL |
| 7. Stop Solution: bottle (ready to use) | 12 mL |
| 8. Wash Concentrate 20X: 1 bottle | 25 mL |

## MATERIALS NOT PROVIDED

1. 1/8” inch hole punch
2. Shaker capable of fixed speed rotation
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 AND STABILITY

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. This kit is designed for Research Use Only. Not for use in Diagnostic Procedures.
3. 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.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. 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

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 100µl of N-TSH Biotin Reagent to all the wells.
4. 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.
5. Cover the microplate and incubate for 60 minutes, on the plate shaker at 900 rpm.
6. Remove the contents of the wells by decantation or aspiration. Make sure all the blood dots are removed at this point. Rinse the wells 5 times with 1X wash buffer. Strike the wells sharply to remove residual wash buffer droplets.
7. Add 100µl of N-TSH Enzyme Reagent of to each well.
8. Cover the microplate and incubate for 60 minutes, on the plate shaker at 900 rpm.
9. Rinse the wells 5 times with 1X wash buffer.
10. Add 100µl of TMB Reagent of to each well.
11. Cover the microplate and put it on the plate shaker at 900 rpm for 15 minutes at room temperature.
12. Add 50µl of stop solution to each well and gently mix until a uniform color, in each well, is obtained.
13. 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 TSH standards (vertical axis) against its concentration in µIU/ml (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 TSH.

| Sample | OD450nm | Conc (µIU/ml) |
|---|---|---|
| 1 | 0.041 | 0 |
| 2 | 0.146 | 10 |
| 3 | 0.257 | 20 |
| 4 | 0.582 | 50 |
| 5 | 1.176 | 100 |
| 6 | 2.351 | 200 |