Datasheet for ABIN996948

Neonatal Thyroxine T4 ELISA Kit

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Overview

Quantity:	96 tests
Target:	Neonatal Thyroxine T4 (NN-T4)
Reactivity:	Chemical
Method Type:	Competition ELISA
Detection Range:	0-25 µg/dL
Minimum Detection Limit:	0 µg/dL
Application:	ELISA

Product Details

Purpose:	The Quantitative Determination of Total Thyroxine Concentration in Human (Neonates) whole blood by a Microwell Enzyme Immunoassay
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	97%
Sensitivity:	0.05 µ/dL

Target Details

Target:	Neonatal Thyroxine T4 (NN-T4)
Abstract:	NN-T4 Products
Target Type:	Chemical

Target Details

Background:	Determination of hypothyroidism within the first few days of birth has been recognized as the single most important diagnostic test in neonates by the American Thyroid Association The need for its early detection and treatment has 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. This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, calibrators, patient specimen, or controls, all made and dried in whole blood are first added to a microplate well. A buffer containing essential ingredients to isolate T4 from blood proteins is added. The blood from the filter paper dots is allowed to elute in the buffer. In the process, T4 (Thyroxine) dissociates from the serum (blood) proteins and binds to the antibody that is immobilized on the inside of the microwells. Excess blood is removed using a wash step. Enzyme-T4 conjugate is added. The enzyme labeled T4 binds to the sites on the antibody left available by the native T4 that came from the sample. After the completion of the required incubation period, excess enzyme conjugate is removed using a wash step. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color. The employment of several references, made in whole blood, of known thyroxine concentration permits construction of a dose response curve (DRC-graph) of activity and con-centration. An unknown specimen's activity can be extrapolated from the DRC.

Application Details

Comment:	Quality Control: Each laboratory should assay external controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained - follow the performance of the supplied reagents. Pertinent statistical methods should be employed - ascertain trends. The individual laboratory should set

	acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used - determine the reason for the variations Limitations of procedure: A. Assay Performance 1. It is important that the time of reaction in each well is held constant for reproducible results. 2. Pipetting of samples should not extend beyond ten (10) minutes - avoid assay drift. 3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used. 4. If more than one (1) plate is used, it is recommended - repeat the dose response curve. 5. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop \ solution. Therefore, the addition of the substrate and the stopping solution should be in the same sequence - eliminate any time deviation during reaction. 6. Plate readers measure vertically. Do not touch the bottom of the wells. 7. Failure - remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and inaccurate results. 8. Use components from the same lot. No intermixing of reagents from different batches. 9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Diagnostic Automation, Inc. may yield inaccurate results. 10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed - ensure compliance and proper device usage. 11. It is important - calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and - perform routine preventive maintenance. Expected ranges of values: Based on the limited number of samples at Diagnostic Automation Inc., and as suggested in the printed literature the normal range for healthy neonates is assigned at 8 - 23 ?g/dl. It is important - keep in mind that any normal range establishment is dependent upon a multiplicity of factors like the specificity of the method, the locale, the population tested and the precision of the method in the hands of technicians. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the technicians using the method with a population indigenous - the area in which the laboratory is located.
Sample Volume:	50 µL
Assay Time:	1 - 2 h
Plate:	Pre-coated

Application Details

Reagent Preparation:	1. Working T4-Enzyme Conjugate Solution Dilute the T4-enzyme conjugate 1:11 with Neo T4 Enzyme Conjugate Diluent in a suitable, clean container. For example, dilute 160 µL of conjugate with 1.6 mL of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within two-three hours for maximum performance of the assay. General Formula: Amount of Buffer required = Number of wells * 0.1 Quantity of T4 Enzyme necessary = # of wells * 0.01 i.e. = 16 x 0.1 = 1.6 mL for NT 4 Conjugate Buffer 16 x 0.01 = 0.16 mL (160µL) for T4 enzyme conjugate 2. Wash Buffer Dilute contents of wash Concenrteate to 1000 mL with distilled or deionized water in a suitable storage container. Store at 2-30° for up to 60 days. Note 1: Do not use the substrate if it looks blue. Note 2: Do not use reagents that are contaminated or have bacteria growth.
Sample Preparation:	The sampling from neonates is performed by lancing the heels of the infants and then spotting enough whole blood on S&S filter paper card (Cat# 903) to fill the marked circle. Allow the filter paper to dry at room temperature overnight away from heat and moisture. Enclose the dry blood specimen (DBS) in a moisture barrier plastic bag with desiccant and send to the laboratory. The specimen should be collected 3-7 days post partum, Physical data including age and weight of the infant, whether a multiple birth, or a premature birth etc should accompany the sample. It is important for the clinician to know these facts in order to properly assess the thyroid status of the infant. The dried blood samples are stable at 2-8 °C for 2-3 weeks if stored in zip-lock, moisture resistant bags with desiccants.
Assay Procedure:	Before proceeding with the assay, bring all reagents and patient samples to room temperature . 1. Assemble the required number of microwells for each calibrator, control and patient sample to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8 °C. 2. Punch out 1/8" blood dot out of each calibrator, control and specimens into the assigned wells. (NOTE: Do not punch blood dots from areas that are printed or that are near the edge of the blood spot). 3. Add 0.100 mL (100 µL of Neo-T4 Elution Buffer to all the wells. 4. Shake the microplate gently for 20-30 seconds to mix. (NOTE: Make sure that all blood dots are fully submerged in the liquid and not stuck to the walls of the microwells). 5. Cover with a microplate cover and rotate for 90 minutes at ambient temperature using a laboratory rotator set @ 150rpm. (Note: see alternative overnight incubation). 6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper. NOTE: Make sure all the blood dots are removed at this point.

	There should be no dots left in the microwells. 7. Add 350 µL of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat four (4) additional times. 8. Add 100 µL of working Neo-T4 Enzyme Reagent to each well. 9. Cover the microplate and rotate for 45 minutes at ambient temperature using a laboratory rotator set @ 150 rpm. (Note: see alternative overnight incubation). 10. Repeat wash step #7. 11. Add 0.100 mL (100 µL of substrate solution to each well. 12. Cover the microplate and incubate for 15 minutes at ambient temperature. No rotation is required for this step. 13. Add 0.050 mL (50 µL of stop solution to each well and gently mix for 15-20 seconds. NOTE: Always add reagents in the same order to minimize reaction time differences between wells. 14. Read the absorbance in each well at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) in a microplate reader. The results should be read within fifteen (15) minutes of adding the stop solution. Alternative overnight procedure: 1. Substitute overnight incubation (12-16h) for the 90 minutes with rotation (Step 5). No rotator is required. Seal the plate(s) with plastic wrap. 2. Substitute 1 hr incubation for the 45 minute incubation with rotation (Step 8). No rotator is required. 3. All other steps remain the same.
Calculation of Results:	A dose response curve is used to ascertain the concentration of Neo-natal T4 in unknown specimens. 1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1. 2. Plot the absorbance for each duplicate serum reference versus the corresponding NEO-NATAL T4 concentration in µg/dL on linear graph paper (do not average the duplicates of the serum references before plotting).

3. Draw the best-fit curve through the plotted points.

4. To determine the concentration of NEO-NATAL T4 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/dL) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.719) intersects the dose response curve at (10.8pg/dL) NEO-NATAL T4 concentration (See Figure 1). Note: Computer data reduction software designed for IEMA (ELISA) assays may also be used for the data reduction. EXAMPLE 1 Sample I.D. Well Number A sigma . Mean (V (q ω d e) 2.528

2.462 0 B1

2.398 Cal B C1

2.082

2.070

1.4 D1

2.059 Cal C E1

1.667

1.641

3.2 F1

1.616 Cal D G1

1.131

1.094

6.5 H1

1.058 Cal E A2 0.648 0.649 13 B2 0.651 Cal F C2 0.386 0.387 25 D2 0.388 Cont - I E2

1.874

1.855

2.3 F2

1.836 Cont - II G2

1.447

1.436

4.3 H2

1.425 Cont - III A3 0.830 0.785

9.8 B3 0.740 Patient C3 0.698 0.719

1.8 D3 0.739 *The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay. φ epsilon Figure 1 5 φ φ Δ < 5 10 15 20 25 MIT4 Values in pg/cll Q. C. PARAMETERS: In order for the assay results to be

considered valid the following criteria should be met.

1. The absorbance (OD) of Calibrator '0' µsigma/dL should be >

1.

3.

2. Four out of six quality control pools should be within the established ranges.

: Precision: The within and between assay precisions of the NEO-NATAL T4 ELISA test system were determined by analyses on three different levels of dried blood controls. The number (N), mean values (X), standard deviation (sigma) and coefficient of variation (C.V.) for each of these controls are presented in Table 2 and Table

3. TABLE 2 Within Assay Precision (Values in mg/dL) Sample N X sigma C.V. Low 20

2.76 0.30

1.9 % Normal 20

5.15 0.45

8.8 % High 20

11.30 0.88

7.8 % TABLE 3 Between Assay Precision (Values in mg/dL) Sample N X sigma C.V. Low 10

2.86 0.24

8.4 % Normal 10

5.24 0.35

6.7 % High 10

11.10 0.88

7.9 % *As measured in ten experiments in duplicate over a ten day period. B. Accuracy The NEO-NATAL T4 ELISA test system was compared with an automated fluorescent methodology. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.5 mg/dL - 46 mg/dL). The total number of such specimens was 370. The least square regression equation and the correlation coefficient were computed for this NEO-NATAL T4 ELISA method in comparison with the reference method. The data obtained is displayed in Table

4. TABLE 4 Method Mean Least Square Regression Analysis Correlation Coefficient This Method (y) 15.63 y = 0.604 +0.941 (x) 0.955 Reference (x) 15.96 Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement. C.

Application Details

Restrictions:	For Research Use only

Handling

Storage:	4 °C
Expiry Date:	12-14 months