



ELEGANCE NEONATAL TSH ELISA KIT

REF 40 450480

Σ 480

REF 40 452400

Σ 2400



WARRANTY

The manufacturer makes no express warranty other than the diagnostic kit will measure the designated analyte when used in accordance with the manufacturer's printed instructions. The use of the diagnostic kit for any other purpose is outside the intended use of this product and is done at the user's own risk.

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INTENDED USE

The *ELEGANCE* Neonatal TSH ELISA has been designed for the quantitative *in vitro* diagnostic measurement of thyroid stimulating hormone (TSH) in the screening of human neonatal blood spots.

PRINCIPLES OF THE ELEGANCE ELISA

The *ELEGANCE* ELISA is an enzyme-linked immunoassay. The TSH is first eluted from the blood spot and at the same time the eluted TSH binds to the anti-TSH antibody on the microwell. The sample antigen is "sandwiched" between the antibody bound to the microwell and the biotinylated antibody reagent. The microwells are washed to remove any unbound material. Streptavidin-peroxidase (Amplification Reagent) is added and binds to the biotinylated antibody at many sites. After washing, the Substrate Solution reacts with any bound peroxidase to produce colour in direct proportion with the amount of sample antigen, which can be calculated from the calibrator curve.

ELEGANCE REAGENTS PROVIDED, STABILITY AND STORAGE

Kit size - 480 tests and 2400 tests (in parentheses).

The kit and all its components, opened or unopened, should be stored at 2-8°C until the listed expiry dates.

Neonatal TSH:

Coated Microwells

1 x 96 wells REF # TNA96

**5 x 96 wells REF # TNA5
(25 x 96 wells REF # TNA25)**

Frame containing microwells coated with anti-TSH antibody. Ready to use.

Neonatal TSH:

Antibody Reagent

1 vial REF # TNB480

(1 vial REF # TNB25)

50 (250) mL biotinylated anti-TSH antibody in a buffered solution containing bovine serum albumin, non-immune animal sera and a blue dye. Contains sodium azide, 0.2% w/v and thiomersal, 0.01% w/v. Ready to use.

Neonatal TSH:

Amplification Reagent

1 vial REF # TNP480

(1 vial REF # TNP25)

50 (250) mL streptavidin-peroxidase (streptavidin from *S. avidinii*) in a buffered solution containing bovine serum albumin and a violet dye. Contains Bronidox L, 0.2% v/v and thiomersal, 0.02% w/v. Ready to use.

Wash Concentrate

1 vial REF # EWC5

(2 vials REF # EWC25)

250 (500) mL of a 15 x concentrated wash solution. Contains thiomersal, 0.09% w/v.

To be diluted before use.

Elution Buffer

1 vial REF # EEB5

(1 vial REF # EEB25)

50 (250) mL buffered solution containing bovine serum albumin. Contains sodium azide, 0.1% w/v.

Ready to use.

Substrate Solution

TMB H

1 vial REF # ETMB5

(1 vial REF # ETMB25)

50 (250) mL 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide in a stabilising solution.

Ready to use.

Stopping Solution

1 vial REF # ESS5

(1 vial REF # ESS25)

30 (124) mL 1M H₂SO₄

Ready to use.

Neonatal TSH: Blood Spots

Calibrators and Controls

1 set REF # ETNS6

(4 sets REF # ETNS7)

Dried human TSH blood spots with 6 calibrators (A-F) and 2 controls (1-2) on filter paper. Ready to use.

PRECAUTIONS AND WARNINGS TO USERS

Handling of specimens and kit components, their use, storage and disposal should be in accordance with any local or national laboratory safety procedures or regulations.

Specimens and Calibrators

The source material of the blood spots have been tested by an approved accredited method for the presence of hepatitis B surface antigen, antibody to hepatitis C and antibody to HIV - 1/2 (AIDS) and have been found to be non-reactive for all. However it is recommended that all samples be handled as if capable of transmitting infectious disease.

Preservatives

The kit contains sodium azide, thiomersal and Bronidox L as preservatives. As reagents contain potentially toxic preservatives, care should be taken in handling, to avoid ingestion or skin contact. Sodium azide may react with lead and copper plumbing to form potentially explosive azides.

Substrate Solution and

Stopping Solution

Avoid any skin contact.

SPECIMEN COLLECTION AND HANDLING

The *ELEGANCE* Neonatal TSH ELISA is intended for use with neonatal heel prick blood samples collected and dried on

filter paper according to NCCLS LA4A Guidelines, where applicable, however cord blood can be used upon validation of process. The filter paper should be Schleicher and Schuell Grade 903 so that the samples are equivalent to the calibrators and controls supplied. Blood from a neonatal heel prick is collected 3-5 days after birth. A blood spot covering one circular sample area on the filter paper is obtained by one application of the filter paper onto a drop of blood flowing from the pricked heel of the baby. The filter paper sample area should be fully covered and soaked through. After collection of the test samples, the filter papers are dried horizontally (2 hours or more). The dry specimens can be stored at 2-8°C. Only blood spots prepared in the above manner should be tested. Unused filter paper should be desiccated at room temperature (20-25°C).

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- * Distilled or deionised water
- * Disc punch (3mm)
- * Forceps
- * Repeating pipette
- * 1L measuring cylinder
- * Absorbent tissue (lint-free)
- * Timer
- * Microtitre plate shaker
- * Microtitre plate washer
- * Microplate reader system.

PROCEDURAL NOTES

Bring all reagents and specimens to room temperature (20-25°C) and mix by gentle inversion prior to use. Duplicates are recommended. Contamination of reagents will lead to poor performance. A calibrator curve should be run with each assay. Prior to first wash, punched discs should be removed either by suction or gentle pin prick. All assay steps should be performed without interruption, but if the wells cannot be filled with Amplification Reagent or Substrate Solution immediately after washing, then the microwells may be left upside down on absorbent lint-free tissue for a maximum of 15 minutes (Amplification Reagent), and 60 minutes (Substrate Solution). Reagents are matched in each kit and therefore reagents from different lot numbers should not be mixed. The photometer and all pipettes used should be calibrated appropriately before use.

Washing

The efficiency of the wash step is vital for good precision. Microwells are washed using an automatic plate washer. Avoid overflows from one well to another.

Quality Control

Control specimens should be run in every assay to ensure correct procedure. Control values should lie within laboratory ranges before assay is approved.

ASSAY PROCEDURE

Preparation of Reagents

Wash Solution

Dilute the wash concentrate 1 in 15 with deionised water. The wash solution can be stored at room temperature (20-25°C) for 12 weeks.

Calibrators and Controls

Exact concentrations determined lot-to-lot are stated on a label for each set of blood spots. To avoid condensation, do not open blood spots before temperature equilibration. Reseal unused blood spot strips in plastic bag and store at 2-8°C.

Protocol

1. Assemble the microwells in the frame according to the number of tests required. Bag and return unused wells to 2-8°C.
2. Place a single 3 mm disc (calibrator, control, specimen) in duplicate into appropriate wells. Avoid the edge of the blood spot when punching the sample.
3. Pipette 100 µL of Elution Buffer (clear) into all wells.
4. Cover microwells with lid and incubate for 60 minutes on a plate shaker at room temperature (20-25°C).
5. Pipette 100 µL of Neonatal TSH Antibody Reagent (blue) into all wells.
6. Cover microwells with lid and incubate for 60 minutes on a plate shaker at room temperature (20-25°C).
7. After incubation, remove discs.
8. Wash the microwells. Aspirate the liquid and rinse each well 4 times with 250 µL of wash solution. After the final wash, invert the microwells and tap firmly on absorbent tissue to remove any remaining wash solution. Ensure that no air bubbles remain in the wells.
9. Pipette 100 µL of Neonatal TSH Amplification Reagent (violet) into each well.
10. Cover microwells with lid and incubate for 10 minutes on a plate shaker at room temperature (20-25°C).
11. After incubation, repeat wash step.
12. Pipette 100 µL of Substrate Solution into all wells.

Timing of the incubation step is measured from the addition of Substrate Solution to the first well.

13. Cover microwells with lid and incubate for 10 minutes stationary at room temperature (20-25°C).

14. Pipette 50 µL of Stopping Solution into all wells in the same timed sequence as for Substrate Solution addition.

15. Carry out an end-point reading at 450 nm and process data as described in the microplate reader user's manual. This reading step should be carried out within 5 minutes of stopping the reaction.

CALCULATION OF RESULTS

Calculation of results can be carried out manually if there is no automatic data reduction. Determine the OD for each well. Plot the calibrator curve using log-log graph paper with concentration of calibrators on the x-axis and OD on the y-axis. The curve may be drawn point-to-point or a curve-fitting routine, such as spline interpolation, may be used. Interpolate the sample values from OD measured from this calibrator curve. Record the value for each sample in mIU/L whole blood TSH. The range of the *ELEGANCE* Neonatal TSH ELISA is from 0 to approx. 250 mIU/L whole blood, but the maximum concentration that can be reported is limited by the linear performance characteristics of the photometer used.

If the OD value of the highest calibrator is above the range of the photometer, then this calibrator must be omitted from the plot of the calibrator curve. Similarly any sample measuring above the range of the microplate reader should be simply noted as greater than the highest acceptable calibrator.

MODEL CALCULATIONS

Endpoint Data	TSH	
ID	Ave OD	(mIU/L W.B.)
0	0.038	
6.11	0.088	
11.3	0.163	
26.0	0.359	
96.0	1.345	
231	3.009	
Sample 1	0.244	17.30
Sample 2	0.955	66.90
Sample 3	0.104	7.42

LIMITATIONS

The test should not be used to detect hypothyroidism in those that have been transfused or been given antibody therapy treatments, or in prematurely born infants.

CALIBRATION

The calibrators and controls supplied in this kit have been prepared from human blood with a haematocrit value of 55% (v/v) and are expressed in mIU/L whole blood (WHO 1st IRP 68/38 for hTSH).

Conversion of standard units may be made using the following relationship:

$$\text{mIU/L serum} = \text{mIU/L whole blood} \times 2.2$$

EXPECTED VALUES

It is recommended that each laboratory establish its own reference range based on a representative sample population. Typically, a cut-off value of 20 mIU/L whole blood is used for infants 3-5 days after birth. Samples measuring lower than the presumptive positive cut-off are considered normal. Actual congenital hypothyroidism cases usually register at least 40 mIU/L whole blood, but have been recorded as low as 25 mIU/L whole blood.

PERFORMANCE CHARACTERISTICS

Intra-assay Precision

Sample	n	Mean ± 2SD (mIU/L whole blood)	%CV
1	20	4.9 ± 0.57	5.8
2	20	14.5 ± 2.00	6.9
3	20	41.7 ± 3.20	3.8

Inter-assay Precision

Sample	n *	Mean ± 2SD (mIU/L whole blood)	%CV
1	11	6.1 ± 0.85	7.0
2	11	16.6 ± 2.10	6.3
3	11	37.8 ± 2.80	3.7

* quadruplicate

Specificity

Analyte	Concentration Assayed	Apparent TSH (mIU/L W.B.)	Crossreactivity
LH	450 IU/L	<1	N.D
FSH	450 IU/L	<1	N.D
hCG	45,000 IU/L	<1	N.D
TSH	57.4 mIU/L	56.8	99%

Accuracy

Recovery was calculated by assaying before and after addition of exogenous analyte.

Sample	Observed TSH (mIU/L W.B.)	Expected TSH (mIU/L W.B.)	% Recovery
1	16.4	16.9	97.0
2	21.8	23.0	94.8
3	64.7	63.1	102.5

Dilution

A sample was diluted in zero serum, assayed and recovery calculated.

Sample	Observed TSH (mIU/L W.B.)	Expected TSH (mIU/L W.B.)	% Recovery
Neat	33.2		
1/2	16.7	16.6	100.6
1/4	8.8	8.3	106.0
1/8	4.5	4.2	107.1

High-dose Hook Effect

No hook effect, characteristic of the assay, was found for test samples with TSH values up to and including the test limit of 4700 mIU/L whole blood.

Sensitivity

The sensitivity of the assay is typically less than 1 mIU/L whole blood. The sensitivity is defined as that concentration of analyte which corresponds to the dose response variable (OD) that is two standard deviations from the mean dose response variable of 28 replicate determinations of the zero calibrator run in three different assays.

ORDERING INFORMATION

The *ELEGANCE* NEONATAL TSH ELISA is manufactured by: Bioclone Australia Pty Limited, 71-73 Railway Parade, Marrickville NSW 2204, AUSTRALIA.
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TECHNICAL SERVICE

Full technical service is available by calling Bioclone on +61 (0) 2 9517 1966 or Freecall 1800 251 138