Neonatal TSH ELISA Kit

For the in vitro determination of Thyroid Stimulating
Hormone from whole blood spot samples

Catalogue Number: EL10012N

96 tests
192 tests
480 tests
960 tests

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

ANOGEN

2355 Derry Road East, Unit 23
Mississauga, Ontario
CANADA L5S 1V6
Tel: (905) 677-9221 or (877) 755-8324
Fax: (905) 677-0023

Email: info@anogen.ca ♦ Web Site: www.anogen.ca

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INTENDED USE

For the quantitative determination of human thyroid stimulating hormone concentration from neonatal whole blood samples collected on Schleicher and Schuell's filter paper. This kit is intended FOR LABORATORY RESEARCH USE ONLY and not for use in diagnostic or therapeutic procedures.

INTRODUCTION

Thyroid-stimulating hormone (TSH) is secreted by the anterior lobe of the pituitary gland and induces the production and release thyroid hormones thyroxin (T4) and triiodothryronine (T3). These thyroid hormones exert a negative feedback on the pituitary. The release of TSH is regulated by TSH-releasing hormone (TRH) produced in the hypothalamus. When there are high circulating levels of thyroid hormone in the blood, less TRH is released by the hypothalamus, so less TSH is secreted by the pituitary. The normal concentration of TSH in the blood is extremely low, but it is essential for maintenance of normal thyroid function.

The determination of serum or plasma levels of TSH is recognised as a sensitive method in the diagnosis of primary and secondary hypothyroidism. Primary Congenital Hypothyroidism (CH) occurs in 1 out of every 3,000 to 7,000 infants and is caused by athyroidism and hypoplasia. If infants are screened for this disorder during their first month, then irreversible mental retardation can be prevented through early diagnosis and proper treatment.

The state of infant's thyroid can be determined by a T4 and TSH combination-screening program. This is the most effective method for the clinician because secondary hypothyroidism may be missed by some TSH screenings and T4 screenings may miss minimal hyperthyroidism. Before starting therapy, a confirmation test should be performed if an infant is thought to be suffering from marginal or borderline hypothyroidism. These determinations should be performed using serum T3, T4, and TSH. Due to infant age, weight, prematurity, and demographic variation concentrations of TSH and T4 have been shown to have some variation. Thus each laboratory must establish its own normal and cut-off values.

Yes Biotech Laboratories has developed a kit using a method of collecting blood spot samples on S&S #903 filter paper and ELISA techniques. This kit can quantitatively determine TSH level in neonates sensitively, accurately, safely and reliably. It is an important and practical tool to determine thyroids state of neonates, thus making it possible to prevent against infant mental retardation.

PRINCIPLE OF THE ASSAY

The Yes Biotech Neonatal TSH quantitative enzyme immunoassay described as a solid phase enzyme linked immunosorbent assay (ELISA). Monoclonal antibodies, specific to TSH, have been bound to the surface of each microplate well. During the course of the assay, a blood sample (collected on filter paper) is added to the microplate wells with Sample Buffer and incubated overnight. After washing the microplate to remove the filter paper and unbound component of the sample, a standardized preparation of horseradish peroxidase-conjugated monoclonal antibody specific for TSH β unit is added to each well and incubated. The TSH, if present in the sample, will bind to the antibody on the coated well and will form an Antibody-TSH-Antibody-HRP "sandwich". The microplate wells are thoroughly washed to remove unbound conjugate.

Next, a TMB (3,3', 5,5' tetramthyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a 15-minute incubation period. Only those wells that contain TSH and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of TSH in the test sample, this neonatal TSH ELISA Kit includes calibration standards and controls. The calibration standards and controls are assayed at the same time as the test samples and allow for the operator to produce a standard curve of optical density versus TSH μIU/mL, serum. Therefore, by comparing the optical density of the samples to this standard curve, the concentration of the TSH in the test samples is then determined.

REAGENTS PROVIDED
All reagents provided are stored at 4°C. Refer to the expiration date on the label.

	96 tests	*192 tests*	*480 test*	*960 tests*
1. **REACTION MICROPLATE** (Part EL12N-1) Pre-coated coated murine monoclonal antibody anti-human TSH	*96 wells*	*192 well*	*480 wells*	*960 wells*
2. **ENZYME CONJUGATE** (Part EL12N-2) Anti-TSH conjugated to horseradish peroxidase with preservative	*12 mL*	*24 mL*	*60 mL*	*120 mL*
3. **TSH STANDARD CALIBRATOR CARD** (Part EL12N-3). One sheet of 6 TSH standards (150, 60, 25, 10, 5, 0 μIU/mL) is prepared on filter paper, which contains a known amount of human TSH calibrated against the WHO 2nd I.R.P of hTSH 80/558.	*1 sheet*	*2 sheets*	*3 sheets*	*5 sheets*
4. **TSH CONTROL CARD** (Part EL12N-4) Three level controls are prepared on filter paper, which contains a known amount of human TSH calibrated against the WHO 2nd I.R.P of hTSH 80/558.	*1 sheet*	*2 sheets*	*3 sheets*	*4 sheets*
5. **SAMPLE COLLECTION CARD** (Part EL12N-5) Schleicher and Schuell's Filter Paper No.903. Sealed in a bag.	*90 sample*	*180 sample*	*468 sample*	*942 sample*
6. **SUBSTRATE A** (Part EL12N-6) Buffered solution with H_2O_2.	*10 mL*	*20 mL*	*50 mL*	*100 mL*
7. **SUBSTRATE B** (Part EL12N-7) Buffered solution with TMB.	*10 mL*	*20 mL*	*50 mL*	*100 mL*
8. **STOP SOLUTION** (Part EL12N-8) 2N sulphuric acid solution (H_2SO_4). Caution: Caustic Material!	*12 mL*	*24 mL*	*60 mL*	*120 mL*
9. **COVER STICK** For avoiding vaporization.	*2*	*4*	*10*	*20*
10. **SAMPLE BUFFER** (Part EL12N-9) PBS Buffer with Animal Serum.	*12 mL.*	*24 mL*	*60 mL*	*120 mL*
11. **Wash Buffer (20X)** (Part EL12N-10)	*60 mL*	*120 mL*	*250 mL*	*500 mL*

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Distilled water
2. Precision pipettes: 50µl, 100µl, 200µl and 1 ml
3. Disposable pipette tips
4. Glass tubes and flasks
5. Microtiter well reader
6. Microtiter plate shaker (optional)
7. Vortex mixer or equivalent
8. Absorbent paper
9. Graph paper
10. Disposable gloves
11. Tweezers
12. Hole Punch (1/8" or 3 mm):

PRECAUTIONS

1. Do not substitute reagents form one kit lot to another. Standards, conjugate, and microplates are matched for optimal performance. Use only the reagents supplied by the manufacturer.
2. Do not use kit components beyond their expiration date.
3. The Reference Standards should be assayed in duplicate on each plate with each run of specimens.
4. Use only reagent grade quality water (ddH_2O).
5. Allow all kit reagents and materials to reach room temperature (18-25°C) before use.
6. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease.
9. To avoid contamination of samples and standards, use clean tweezers when handling, not hands.
10. To avoid cross contamination, wash and clean hole punch before punching a new test sample.
11. Each Standard Calibration Card blood spot may be punched approximately 5-6 times, if done carefully. This is important to remember for partial plate usage. When punching from the provided standard calibrator card make sure the provided standards are punched from low to high concentration (starting at 0 µIU/ml).

SAMPLE COLLECTION AND STORAGE

1. Within 3 days collect a blood sample from the heel of the infant
2. Clean the heel of the infant with soap and water. Wipe area dry. Use alcohol on the area. Air dry.
3. With a lancet (<2.4 mm in length) that has been properly sterilized, prick the heel of the infant once and wipe away the initial drop of blood. After another drop has formed use the sample collection card provided in the test kit to collect the infant' s blood on the card. Do this by gently pressing the drop of blood into the centre of the pre-printed circle on the sample collection card. Do not tear the filter paper surface. To avoid haemolysis and dilution of the blood sample do not exert excessive pressure during collection.
4. Let sample card air dry, for no less than 3 hours at room temperature (18°C to 25°C). Place card in a clean area and away from direct sunlight.

5. Within 24 hours, place each sample in its own individual paper envelope. Place in a moisture-proof bag at 2-8°C for short-term storage and -20°C for long-term storage. (NCCLS publication LA4-A2)

PREPARATION OF REAGENTS

All reagents should be brought to room temperature (18-25°C) before use.

Substrate Solution: Substrate A and B should be mixed together in equal volumes up to one hour before use. This table is for your reference.

Wells Used	Substrate A (ml)	Substrate B (ml)	Substrate Solution (ml)
16 wells	1.5	1.5	3.0
32 wells	3.0	3.0	6.0
48 wells	4.0	4.0	8.0
64 wells	5.0	5.0	10.0
80 wells	6.0	6.0	12.0
96 wells	7.0	7.0	14.0

Wash Buffer: Dilute 1 volume of Wash Buffer (20X) with 19 volumes of distilled or deionized water. Wash Buffer is stable for 2 weeks at room temperature or 1 month at 2-8°C. Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.

2. Dispense standards, samples, and controls by punching one 3mm blood spotted filter paper into the correct wells (in duplicate).

3. Add 100µl Sample Buffer to each well. Mix them by gentle tapping the plate for 20 seconds. Cover and incubate for 1 hour at 4°C. Mix them by gentle tapping the plate for another 20 seconds. Then incubate the plate overnight at 4°C. <u>It is recommended to incubate plate for 20 hours at 4°C.</u>

4. Remove incubation mixture (including blood spotted filter paper) with wash buffer by flicking the plate contents into waste container.

5. Wash the Microtiter Plate using one of the specified methods indicated below:

   <u>Manual Washing</u>: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer(1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. *It is recommended that a soaking time of 15 seconds be used between washes.*

Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer(1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350µl/well/wash (range: 350-400µl). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds be used between washes.*

6. Add 100µl of Enzyme Conjugate to each well. Cover plate and incubate for 1 hour at 37°C.

7. Prepare Substrate Solution (see preparation of reagents) no more than 15 minutes before end of second incubation.

8. Repeat wash procedure as described in step 5.

9. Add 100µl of Substrate Solution into each well. Cover and incubate 15 minutes at 37°C.

10. Stop reaction by adding 100µl of Stop Solution to each well. Mix well.

11. Read the absorbency at 450 nm within 30 minutes.

CALCULATION OF RESULTS

1. There are two recognised TSH value expression methods, one is TSHµIU/ml serum and another is TSHµIU/mL whole blood. **The data from this Kit are expressed as TSH µIU/mL serum.*** **To convert µIU/mL serum units to µIU/mL blood units, divide by 2.22.**

2. Calculate the mean absorbency value (OD_{450}) for each set of standards, controls, and samples. All absorbency values, for the standards and the samples, are subtracted by the mean value of the 0 µIU/ml TSH Standard.

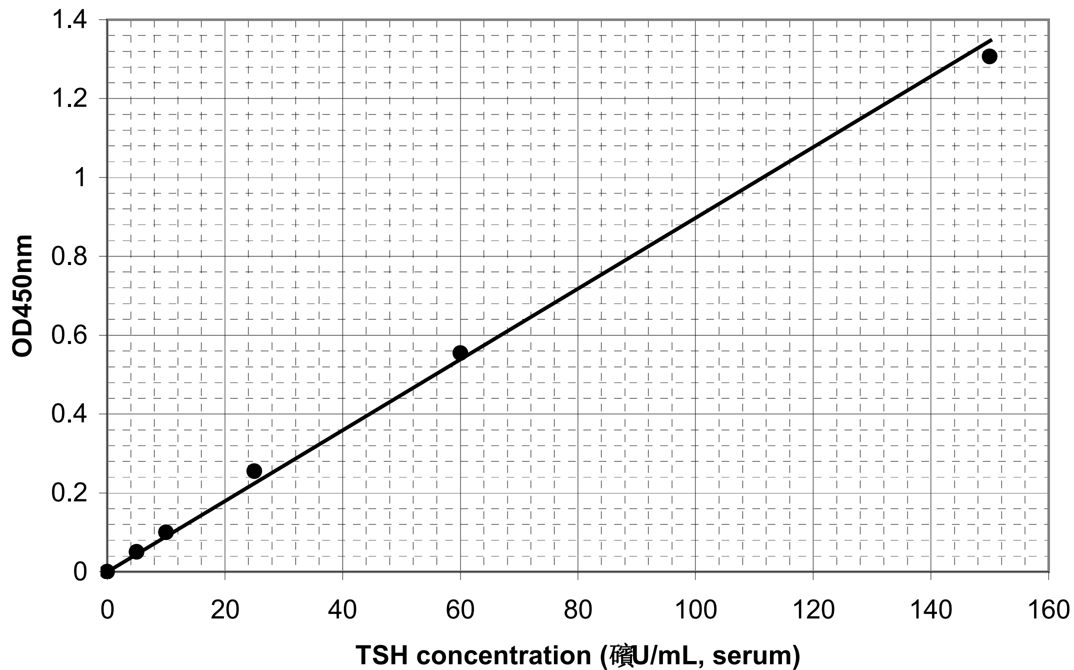
3. Construct a standard curve by plotting the absorbency obtained from each reference standard against its concentration of TSH in µIU/ml on linear graph paper. The absorbency is plotted on the vertical or Y-axis and concentration on the horizontal or X-axis.

4. To determine the TSH concentration for each sample, first locate the mean absorbency value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding TSH concentration.

EXAMPLE OF STANDARD CURVE

1. Typical Data:
   Results of a typical standard run of a neonatal TSH ELISA are shown below.
   The standard curve covers a dynamic range from 0.77µIU/ml to 150µIU/ml. The following example is for the purpose of *illustration only*, and should not be used to calculate unknowns. Each user should obtain their own standard curve. (N/A = not applicable)

Standard (μIU/ml, serum)	ABS450	Mean	Zero Standard Subtracted	Assayed Range (μIU/ml, serum)	TSH (μIU/ml, serum)
0	0.061 0.054	0.058	0	N/A	N/A
5	0.111 0.105	0.108	0.050	N/A	N/A
10	0.157 0.160	0.158	0.100	N/A	N/A
25	0.318 0.307	0.313	0.255	N/A	N/A
60	0.631 0.594	0.613	0.555	N/A	N/A
150	1.364 1.366	1.365	1.307	N/A	N/A
Control I	0.165 0.166	0.166	0.108	6.5 - 15.0	12
Control II	0.272 0.268	0.270	0.212	14.0 - 29.0	23
Control III	0.504 0.499	0.502	0.444	25.0 - 65.0	48



PERFORMANCE CHARACTERISTICS

1. *Intra-assay precision:*
   Within-run precision was determined by replicate determinations of three different test samples of known concentration in one assay. The mean precision of intra-assay is 6.6%.

Sample	*1*	*2*	*3*
n	16	16	16
Mean (μIU/ml, *serum*)	21.75	71	146.7
Standard Deviation	1.46	4.98	9.03
%CV	6.7	7.0	6.2

2. *Inter-assay precision:*
   Between-run precision was determined by replicate measurements of three different test samples of known concentration in 16 different assays. The mean inter-assay precision is 10.5 %.

Sample	*1*	*2*	*3*
n	16	16	16
Mean (μIU/ml, *serum*)	20.8	69.7	137.9
Standard Deviation	2.65	5.99	13.87
%CV	12.8	8.6	10.1

3. *Recovery:*
   The recovery of THS spiked to three levels in a pooled whole blood (human, TSH free) throughout the range of the assay in various matrices was evaluated.

Added (μIU/ml, serum)	*Measured* (μIU/ml, serum)	*%Recovery*
20	21.75	108.8
70	71.0	101.4
140	146.7	104.8

4. *Sensitivity:*
   The minimal detectable concentration of TSH was determined by adding two standard deviations to the mean optical density value of 16 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose using a standard curve is 0.77μIU/ml.

5. *Specificity:*
   This kit exhibits no significant detectable cross-reactivity with HCG, hLH, hFSH, hProlactin, and hGH. Interference by HCG, hLH, hFSH, hProlactin, and hGH were measured by adding physiological amounts of each hormone into each blood sample.

Tested hormones	Added concentration of hormones	Measured TSH (μIU/mL *serum*)
HCG	20, 000 mIU/mL	0.25
hLH	500 mIU/mL	1.5
	100 mIU/mL	0.3
hFSH	500 mIU/mL	1.0
	100 mIU/mL	0.2
hProlactin	500 ng/mL	0.2
hGH	500 ng/mL	0

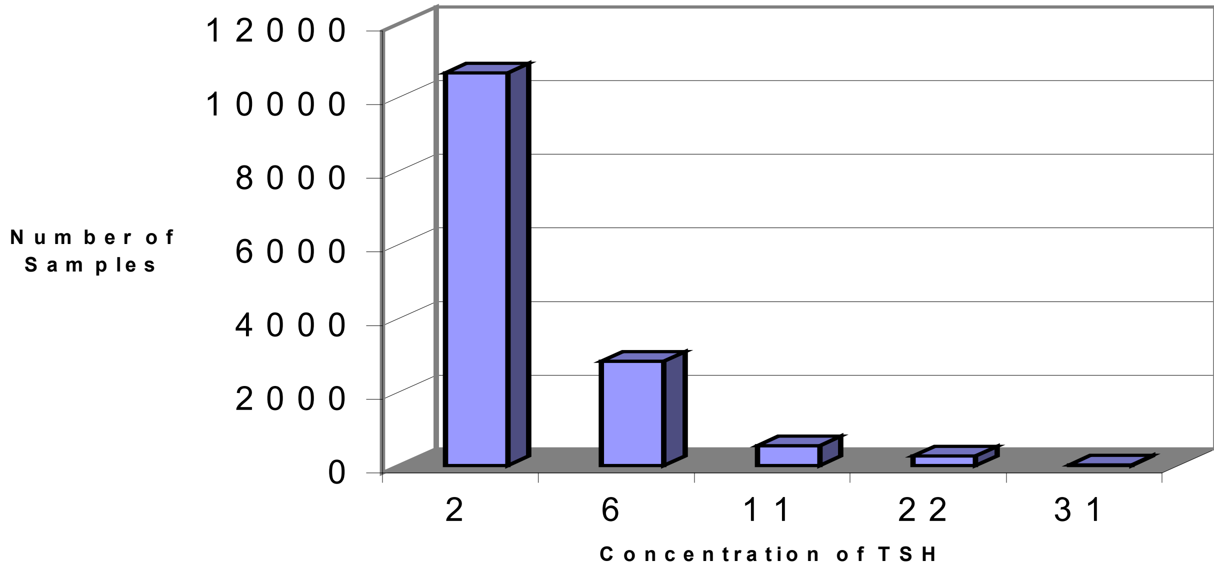
6. *Hook effect:*
   In this assay, no hook effect is observed up to 16, 200 μIU/mL serum.

7. *Calibration:*
   The TSH Standard use in this kit is calibrated against WHO 2nd I.R.P of hTSH 80/558.

8. *Accuracy:*
   Accuracy was studied using CDC (Atlanta, USA) Quality Control samples.

Sample No.	CDC Enriched Value (TSH μIU/ml, serum)	Yes NTSH Kit Measured (TSH μIU/ml, serum)
0110166	25	28.5
0120702	40	48.2
0130913	80	91.0

9. *Clinical Evaluation*:
   12298 new born infant whole blood samples were collected on Sample Collection Card (S&S Filter Paper #903) and tested with Yes Neonatal TSH EIA Kit.



In this Clinical Evaluation of Yes Biotech Neonatal TSH ELISA Kit, 12298 new-born infant whole blood samples submitted a distribution pattern and normal range for our evaluation. The frequency distribution analysis of neonatal TSH showed that 86.7% of new-borns were 0-2.2µIU/mL and the 97.5% neonatal TSH cut-off was around 10µIU/mL. It is, however, recommended that each laboratory establishes its own cut-off value and re-tests those samples their values are higher or close to cut-off value.

10. *Cautions:*

a) All samples must be collected on Sample Collection Card (Schleicher and Schuell's Filter Paper #903).
b) The user must strictly follow the protocols outlined in this insert to obtain reliable results. No modifications or changes should be made to the assay protocols.
c) During the incubation period make sure all blood spots are within the conjugate to ensure accurate and reliable results.
d) TSH concentrations may be affected by demographic variations, infant prematurity, age, weight, and twinning.
e) If test samples generate a value higher than the highest standard then the concentration of TSH is > 150 µIU/ml.

11. *Limitations of the Procedure:*

a) It is recommended that a qualified and trained laboratory technician perform the assay.
b) In Northern America, the second testing at 2-6 weeks of age may be required to detect all cases.
c) Although Yes NTSH ELISA Kit is very accurate in detecting neonatal TSH, but combined tests for low T4 and high TSH has greater specificity than either test alone. As all diagnostic tests, a definitive clinical diagnosis should not base on the results of a single test, can only be made by physician after all clinical and laboratory finding have been evaluated.

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