

Neo-TSH (NTSH) ELISA Kit

Neo-TSH

960 Tests Kit

Enzyme Immunoassay for the
 Quantitative Determination of
 Neonatal Thyroid Stimulating Hormone (TSH)
 Concentration from Dry Blood Spot
 For In Vitro Diagnostic (IVD) Use Only
 Catalogue No. PT-Neo-TSH-960

Introduction

Congenital hypothyroidism is a dangerous disease which affects 1 in 3000 to 4000 infants. If not screened during their first 2-6 days of birth, the irreversible brain damage or mental retardation can not be prevented. The Neo-TSH kit is developed to determine, TSH concentration from newborn's blood sample collected on filter paper (S&S 903)

Principle of the assay

The Neo-TSH ELISA test is based on the principle of a sandwich enzyme-linked immunosorbent assay (Sandwich ELISA). The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Blood sample is added to the microplate with extraction buffer and shake for 2 hours at room temperature or incubated overnight. After washing the plate to remove filter paper and unbound components of the sample, the second antibody which is peroxidase conjugated monoclonal antibody specific for TSH is added to form an Antibody-TSH-Antibody-HRP "sandwich". After incubation, the wells are washed with washing solution to remove unbound labeled antibodies. A solution of TMB is then added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the stop solution and measured spectrophotometrically at 450 nm. The concentration of TSH is directly proportional to the color intensity of the test sample.

Materials provided with the kit

- | | |
|--|-------------|
| 1. Microplate: pre coated monoclonal antibody (Anti-h TSH) | 10×96 wells |
| 2. Extraction buffer: Phosphate buffer saline Solution with 0.05 % Kathon CG as preservative
pH = 7.3 , Ready to use | 2×60 ml |
| 3. Wash buffer (10X) : Should be diluted to 500 ml with distilled water before use | 5×100 ml |
| 4. Enzyme conjugate : Anti-hTSH conjugated to horseradish peroxidase with preservative, ready to use | 2×60 ml |
| 5. Chromogen-substrate solution: Contains Tetra Methyl Benzidine (TMB) and hydrogen peroxide, ready to use | 3×60 ml |
| 6. Stop solution : HCl acid, 1N
caution : caustic material | 2×60 ml |
| 7. TSH calibrators : 6 concentrations (0, 7.5, 15, 30, 60 and 105 µIU/ml) of hTSH in whole blood placed on filter paper (S&S 903) calibrated against the WHO 2nd I.R.P. of hTSH 80/558 standard (Keep the calibrators at -20°C for longer storage) | 5×6 Spots |
| 8. TSH controls : Two level controls of known amount of TSH on filter paper (check their labels) | 5×2 Spots |
| 9. Cover stick: For avoiding vaporization | 10 Sheets |
| 10. Disposable Pippete tips | 100 Tips |

Materials required but not provided

- Microplate reader (450 nm) -630 as reference filter
- Orbital or ELISA shaker
- Precision pipettes 100 µl
- Hole puncher (3mm)
- Disposable gloves
- Absorbent towel
- Graph paper
- Distilled water

General Information

- Do not mix kit reagents from different lot numbers.
- All kit components must be used only in their original kit.
- All reagents obtained from human sources are negative for HBs Ag, HCV and HIV. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.



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Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.

Sample collection and storage

Within 3 days after birth, collect a blood sample from the heel of the infant as follows:

- 1- All samples must be collected on Sample Collection Card (Schleicher and Schuell's Filter Paper #903).
- 2- Clean the heel of the infant with soap and water wipe area dry.
- 3- Use alcohol (70% isopropanol) on the area and air dry.
- 4- With a lancet (2.4 mm in length), prick the heel once and wipe away the initial drop of blood.
- 5- After another drop is formed; use the sample collection card to collect the infant's blood on the card.
- 6- Do this by gently pressing the drop of blood in to the center of the pre-printed circle on the sample collection card. *Do not tear or scratch the filter paper surface. To avoid hemolysis and dilution of the blood sample do not exert excessive pressure during collection.*
- 7- Let sample card air dry, for no less than 3 hours at room temperature (22-28°C). Place card in a clean area and away from direct sunlight and heat.
- 8- Within 24 hours, place each sample in individual paper envelope.
- 9- Place in a moisture-proof bag at 2-8°C for short-term and -20°C for long-term storage.

Reagents Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: dilute concentrated wash solution 1:10 with distilled water (i.e. 50 ml of wash solution should be diluted to 500 ml) before use.

Assay procedure

To obtain reliable results, the user should be a qualified and trained for the kit procedure. 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.

- 1- Secure the desired number of coated wells in the holder.
- 2- Dispense standards, samples and controls by punching a 3 mm diameter of blood spotted on filter disk in to the correct wells (Do it in duplicate). In the case of mistake, take care to do not scratch the well for removing disk/s.

Note: Don't punch blood spot near to its edge or areas containing print.

- 3- Add 100 µl of extraction buffer. Be sure the whole disk is soaked in buffer and no bubble is produced in wells.
- 4- Cover the plate with cover stick and mix by gently tapping for 30 seconds and incubate for 2 hours at room temperature with shaking (~ 60 rpm) or 15 hour at 4°C (keep cover stick clean for the whole procedure). Note: During the incubation period make sure all blood spots are within the extraction buffer to ensure accurate and reliable results.
- 5- Remove incubation mixture (including blood spotted filter paper) by flicking the plate contents into the waste container. Those papers which remain in place should be removed by working washing solution.
- 6- Wash the plate by manual or automatic washing. In the manual technique flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each time with 300 µl of working wash solution). Strike the wells sharply onto absorbent paper or paper towel to remove all residual wash solution droplets. In the automated washing system adjust your washer to aspirate as much liquid as possible and set fill volume at 300 µl/ well/wash for 5 times. After final wash, invert plate and blot dry by tapping plate on to absorbent towel until no moisture appears. It is recommended that in both techniques a soaking time of 10 seconds and shaking time of 5 seconds be used between washes.
- 7- Add 100 µl of enzyme conjugate to each well, cover plate and incubate for 1 hour at room temperature.
- 8- Repeat wash procedure as step 6.
- 9- Add 100 µl of chromogen (substrate) solution (TMB) into each well. Cover and incubate 15 minutes at room temperature and dark.
- 10- Add 100 µl of stop solution to the wells to stop reaction.
- 11- Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available)

Calculation of results

- Calculate mean absorbance value of standards and samples at 450 nm. (Use 630 nm filter as reference filter if it's available)
- Construct a point to point standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in µIU/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
- Determine the TSH concentration for each sample on standard curve.



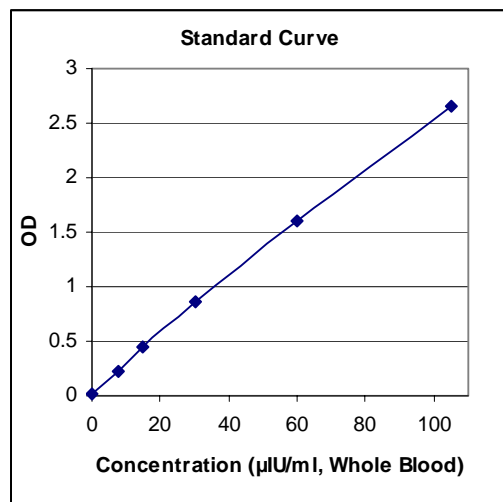
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- Results of a typical standard run of a neonatal TSH ELISA are shown in table 1.
- If test samples generate a value higher than the highest standard then the concentration of TSH should be reported as > 105 $\mu\text{IU/ml}$.

Example of typical standard curve

Standard ($\mu\text{IU/ml}$, WB)	Mean Abs Readings (450 nm)
0.0	0.01
7.5	0.22
15.0	0.44
30.0	0.87
60.0	1.79
105	2.65

Table. 1 Standard values and related mean OD



Graph. 1. Standard curve

Please note: example is for the purpose of illustration only, and should not be used to calculate unknowns. Users should obtain their own standard curve.

Reference Interval

TSH concentrations may be affected by demographic variations, infant prematurity, age, weight, and twinning. To determine reference interval for TSH, 500 new born infant whole blood samples were collected on Sample

Collection Cards (S&S Filter Paper #903) and tested with Neonatal TSH ELISA Kit and their distribution pattern as well as reference interval identified.

The frequency distribution analysis of neonatal TSH showed that in 86.7% of new-born infants TSH range was 0-2.2 $\mu\text{IU/mL}$ and in the 97.5% neonates TSH cut-off was about 10 $\mu\text{IU/ml}$.

However, it is recommended that each laboratory establishes its own cut-off value and re-tests those samples which their values are higher or close to cut-off value. (Our reference interval determination studies in Turkey on about 6000 neonatal blood samples showed 12-16 $\mu\text{IU/ml}$ as gray zone which mean the samples are required to re-test and more than 16 $\mu\text{IU/ml}$ should be considered as positive.)

Performance characteristics

1- Within-run precision was determined by replicate determination of four different test whole samples blood samples of known concentration in one assay.

Sample	1	2	3	4
Replicates	12	12	12	12
Mean ($\mu\text{IU/ml}$ whole blood)	5.1	8.6	17.9	30
Standard Deviation	0.6	0.7	1.3	2.0
% CV	11.8	8.1	7.3	6.7

2. Between-run precision was determined by replicate measurements of four different test samples of known concentration in 12 different assays.

Sample	1	2	3	4
Replicates	12	12	12	12
Mean ($\mu\text{IU/ml}$ whole blood)	4.7	8.8	18.6	31.7
Standard Deviation	0.6	0.9	1.3	2.3
% CV	12.8	10.2	7.0	7.3

3- Sensitivity the minimal detectable concentration of TSH was determined by adding two standard deviations to the mean optical density value of 12 replicates zero standards, calculating the corresponding concentration from the standard curve. The minimum detectable dose using a standard curve is 1.2 $\mu\text{IU/ml}$.



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4- Specificity this kit exhibits no significant detectable cross-reactivity with hCG, hLH and hFSH hormones. Interference was studied by adding physiological amounts of each hormone into each blood sample.

Tested hormones added	Concentration of hormones (IU/L)	Measured TSH (μ IU/ml Whole blood)
hFSH	1000	< 1.2
	100	< 1.2
	10	< 1.2
hLH	1000	< 1.2
	100	< 1.2
	10	< 1.2
hCG	100000	< 1.2
	10000	< 1.2
	1000	< 1.2

5-Hook effect, no hook effect is observed up to 1500 μ IU/ml TSH in whole blood.

6. Calibration:

The TSH Standards used in this kit is calibrated against WHO 2nd I.R.P of hTSH 80/558.

7. Proficiency test:

Accuracy was studied using DGKL (GERMANY) Quality Control samples (TS1/08).

Sample No. TS1/ 08	DGKL Enriched Value (TSH μ IU/ml, Whole blood)	NTSH Kit result (TSH μ IU/ml, Whole blood)
9999751-1	10.5 (6.30 -14.7)	10.3
9999751-2	4.70 (2.30 -7.10)	3.4
9999751-3	26.2 (15.7 -36.7)	29.2
9999751-4	17.0 (10.2 -23.8)	15.8

8. Limitations of the Procedure:

- a) It is recommended that a qualified and trained laboratory technician perform the assay.
- b) The second testing at 2-6 weeks of age may be required to detect all cases.
- c) Although 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. Like other diagnostic tests, a definitive clinical diagnosis should not just rely on the results of a single test. All clinical and laboratory finding should also have been evaluated.

REFERENCES

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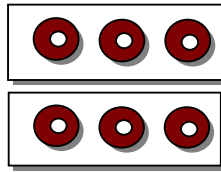
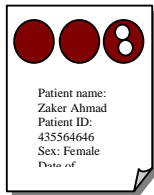
Neo-TSH Test Procedure

1. Punching the:

SAMPLE

CONTROLS

STANDARDS



Duplicate sample into appropriate wells

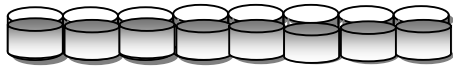


2. Add 100 µl of extraction buffer to all wells and cover with cover stick.

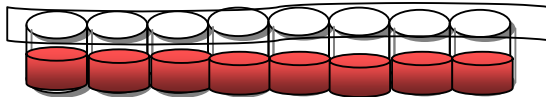


3. Shake (~60 rpm) for 2h at room temperature or 15 h at 4°C

4. Remove incubation mixture (including blood spotted filter paper) and wash them 5 times (300 µl each)



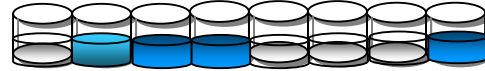
5. Add 100 µl of enzyme conjugate into the wells, cover it and incubate 1 hour at room temperature.



Light Red color

6. Wash as stage 4.

7. Add 100 µl of colorless chromogen substrate solution into the all wells. Incubate 15 min. in dark and room temperature



Blue color

8. Add 100 µl of stop solution into each well and read at 450 nm. (Use 630 nm filters as reference filter if it's available)



Colorless/ yellow color