Instruction for use

A solid-phase enzyme immunoassay for the quantitative determination of TSH in human newborn blood specimens dried on filter paper

1. INTENDED USE

A solid-phase enzyme immunoassay for the quantitative determination of TSH in newborn blood specimens dried on filter paper.

This kit is designed for measurement of TSH in newborn blood specimens dried on filter paper. For possibility of use with other sample types, please, refer to Application Notes (on request). The kit contains reagents sufficient for 96 determinations and allows to analyze 42 unknown samples in duplicates.

2. SUMMARY AND EXPLANATION

TSH-Neo ELISA is intended for the quantitative determination of Thyroid Stimulating Hormone (TSH) in newborn blood specimens dried on filter paper and used as a screening test for congenital hypothyroidism in babies.

TSH is a glycoprotein with MM ca. 30 kDa which is secreted by the anterior lobe of the pituitarty gland. TSH molecule is combined from two non-covalently bound polypeptide chains: alpha-chain and beta-chain. Specificity and biological activity of TSH is caused by its beta-chain. Congenital hypothyroidism is a hereditary pathology expressed in altered thyroid function and found with prevalence ca 1:3500 – 1:4000.

Congenital hypothyroidism is characterized by a decreased secretion of thyroid hormons – T3 and T4, this in turn upregulating TSH secretion and leading to elevated TSH levels in blood.

Screening of newborns with congenital hypothyroidism is based on cut-off TSH value. If TSH value obtained is higher that the cut-off, it indicates a possible pathological state and requires additional laboratory investigations. To confirm the diagnosis, quantification of TSH and T4 in serum is required.

3. PRINCIPLE OF THE TEST

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal antibodies to human TSH β-chain. Antigen from the paper disc is eluted and captured by the antibodies coated onto the microwell surfaceUnbound material is removed by washing procedure. Second antibodies – murine monocnoclonal antibodies to human TSH β-chain, labelled with peroxidase enzyme, are then added into the microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

4. WARNINGS AND PRECAUTIONS

4.1. For professional use only.

4.2. This kit is intended for in vitro diagnostic use only.

4.3. INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are not present in the reagents of this kit. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

4.4. Avoid contact with stop solution containing 5,0% H2SO4. It may cause skin irritation and burns.

4.5. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.

4.6. Do not use the kit beyond the expiration date.

4.7. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.

4.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

4.9. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

4.10. Do not mix reagents from different lots.

4.11. Replace caps on reagents immediately. Do not swap caps.

4.12. Do not pipette reagents by mouth.

4.13. Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.

4.14. Safety Data Sheet for this product is available upon request directly from XEMA Co., Ltd.

4.15. The Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

5. KIT COMPONENTS

5.1. Contents of the Kit

	Symbol	Description		Qty	Units	Colour code	Stability of opened/diluted components
1	SORB MTP	TSH-Neo EIA strips, 8x12 wells	polystyrene microwells coated with murine monoclonal antibody to beta TSH	1	pcs		until exp.date
2	CAL 1–6	Calibrator set, 2 pcs each. The set contains 6 calibrators: 0; 10; 25; 50; 100, 250 µIU/ml human blood specimens dried on filter paper	human blood specimens dried on filter paper Concentrations are calibrated against the WHO 2nd IRP (80/558) of TSH. Concentrations may slightly differ from the above mentioned values, the exact values being indicated on the package	6	pcs		2 months
3	CONJ HRP	Conjugate, 11 ml	aqueous solution of murine monocnoclonal antibodies to human TSH β-chain coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0,1% phenol as preservative and red dye	1	pcs	red	until exp.date
4	DIL	EIA Sample buffer 22 ml	phosphate buffered saline with casein from bovine milk and detergent (Tween-20), contains 0,1% phenol as preservative; contains blue dye	1	pcs	blue	until exp.date
5	SUBS TMB	Substrate solution, 11 ml	ready-to-use single-component tetramethylbenzidine (TMB) solution.	1	pcs	colourless	until exp.date
6	BUF WASH 21X	Washing solution concentrate 21x, 44 ml	aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative	1	pcs	colourless	Concentrate - until exp.date Diluted washing solution – 1 month at +2…+8 °C or 5 days at RT
7	STOP	Stop solution, 11 ml	5,0% vol/vol solution of sulphuric acid	1	pcs	colourless	until exp.date
8	N003	Plate sealing tape		2	pcs		N/A
9	K201NI	Instruction TSH-Neo EIA		1	pcs		N/A
10	K201NQ	QC data sheet TSH-Neo EIA		1	pcs		N/A

5.2. Equipment and material required but not provided
- Distilled or deionized water;
- Automatic or semiautomatic multichannel micropipettes, 100–250 µl, is useful but not essential;
- Calibrated micropipettes with variable volume, range volume 25–250 µl;
- Microtiter plate shaker. Shaking should be medium to vigorous. Longitudinal shaking approximately 200 strokes/min, oscillations 600–800/min
- Calibrated microplate photometer with 450 nm wavelength and OD measuring range 0–3.0.
- Puncher to obtain specimen paper discs from dried blood spots.

5.3. Storage and stability of the Kit

Store the whole kit at 2 to 8 °C upon receipt until the expiration date.

After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

6. SPECIMEN COLLECTION AND STORAGE

This kit is intended for use with blood specimens dried on filter paper. Blood is taken with a sterile scarifier from a finger or a heel. It is essential that blood spots are collected by application of a single drop of blood on a special blank printed on a filter paper. Layering of successive drops will lead to elevated results. It is also important that the blood penetrates the filter paper from one side to the other to produce just the same spot from the both sides. The obtained pots should be dried in horizontal position for 2–3 hrs at RT (+18...+25 °C) avoiding direct sunlight.

Blood spot size should be nlt 12 mm diameter; nlt three such spots should be obtained.

Blood applied on a filter paper should not contain EDTA – it may cause false results.

7. TEST PROCEDURE

7.1. Reagent Preparation
- All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18…+25 °C) before use.
- All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
- It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
- Prepare washing solution from the concentrate BUF WASH 21X by 21 dilutions in distilled water.

7.2. Procedural Note:

It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

7.3. Assay flowchart

See the example of calibration graphic in Quality Control data sheet.

7.4. Assay procedure

1	Put the desired number of microstrips into the frame; allocate 12 wells for the calibrators CAL 1–6 and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.
2	Pipet 200 µl of EIA Sample buffer into each well.
3	Using disc puncher, obtain discs of ca. 3.2 mm diameter from calibrators and unknown specimens blood spots. IMPORTANT: the discs obtained should be completely immersed with blood from both sides – otherwise, the results may be false.)
4	Dispense in duplicates discs from calibrators, controls and unknown specimens in the appropriate wells. Cover the wells by plate adhesive tape.
5	Incubate overnight (12–18 hrs) at +18…+25 °C and continuous shaking at 600 rpm
6	Prepare washing solution by 21x dilution of washing solution concentrate BUF WASH 21X by distilled water. Minimal quantity of washing solution should be 250 µl per well. Wash strips 5 times
7	Aspirate the contents of the wells by disc remover. Wash strips 5 times.
8	Dispense 100 µl of CONJ HRP into the wells. Cover the wells by plate adhesive tape.
9	Incubate 60 minutes at +18…+25°C and continuous shaking at 600 rpm
10	Wash the strips 5 times.
11	Dispense 100 µl of SUBS TMB into the wells
12	Incubate 10–20 minutes at +18…+25 °C
13	Dispense 100 µl of STOP into the wells.
14	Measure OD (optical density) at 450 nm.
15	Set photometer blank on first calibrator
16	Apply point-by-point method for data reduction.

8. QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

9. CALCULATION OF RESULTS

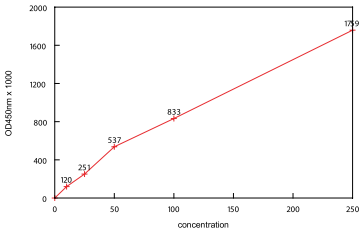
9.1. Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.

9.2. Plot a calibration curve on graph paper: OD versus TSH concentration.

9.3. Determine the corresponding concentration of TSH in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

9.4. Below is presented a typical example of a standard curve with the XEMA Co. Not for calculations!

Calibrators	Value	Absorbance Units (450 nm)
CAL 1	0 µIU/ml	0.097
CAL 2	10 µIU/ml	0.217
CAL 3	25 µIU/ml	0.348
CAL 4	50 µIU/ml	0.634
CAL 5	100 µIU/ml	0.930
CAL 6	250 µIU/ml	1.856



10. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own cut-off value for TSH-Neo. Based on data obtained by XEMA, the following cut-off is recommended: 20 µIU/ml blood (equivalent to 44 µIU/ml serum with hematocrit value 50-55%). TSH-Neo value higher than 20 µIU/ml is indicative of possible thyroid pathology which requires additional laboratory investigations. All samples with values higher than the cut-off should be retested to exclude false-positive results.

If the retested sample gives elevated results again, a new blood specimen should be taken from the same newborn.

If the result is within 20-100 µIU/ml, the blood sample is taken on filter paper blank and dried blood specimen should be investigated.

If the result is higher than 100 µIU/ml, a serum sample should be obtained and used for quantitative determination of TSH, T4 and T3.

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical specificity / Cross reactivity

Analyte	Cross-reactivity, % wt/wt
HCG	<0.1
LH	<0.1
FSH	<0.1

11.2. Analytical sensitivity

Sensitivity of the assay was assessed as being 3 µIU/ml.

11.3. Repeatability

RSD, % for the same blood specimen is nmt 15%