TSH neonatal screening ELISA

Enzyme immunoassay for the in-vitro-diagnostic quantitative determination of Thyroid Stimulating Hormone (TSH) in human newborn blood spot samples. For neonatal screening of congenital hypothyroidism (CH).

REF RE55105 / RE55109

Σ 480 / 2400

2-8°C

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For research use only. Not for use in diagnostic procedures.
1. **INTENDED USE**
Enzyme immunoassay for the *in-vitro-diagnostic* quantitative determination of Thyroid Stimulating Hormone (TSH) in human newborn blood spot samples. For neonatal screening of congenital hypothyroidism (CH).

2. **SUMMARY AND EXPLANATION**
Human thyrotropin (thyroid stimulating hormone, hTSH) is a glycoprotein synthesised in and secreted from the anterior lobe of the pituitary gland. It regulates and stimulates the thyroid gland to produce T4 and T3. The deficiency of thyroid hormones stimulates hTSH secretion through a negative feedback mechanism. The level of hTSH is also influenced by hTSH-releasing hormone (TRH), a tripeptide produced by the hypothalamus.

Failure in any portion of a newborn’s developing thyroid gland system may result in congenital hypothyroidism (excess of TSH). Congenital hypothyroidism (CH) is one of the most common and preventable causes of mental retardation and occurs in approximately 1 in 4,000 infants worldwide. Yet due to the lack of specific symptoms and signs in the early neonatal period, clinical diagnosis occurs in <5% of newborns with CH. Without prompt treatment, irreversible mental retardation, growth failure and a variety of neuropsychological deficits are inevitable. Clearly, these features of CH show that diagnosis and treatment must occur as early as possible. Since the development of pilot screening for congenital hypothyroidism in Quebec and Pittsburg in 1974, newborn screening for congenital hypothyroidism has become routine in all developed countries and most of Eastern Europe, and is under development in many developing countries. [1, 2, 3, 4, 5].

3. **TEST PRINCIPLE**
TSH neonatal screening ELISA is a “sandwich” type of solid-phase enzyme immunoassay, based on two monoclonal antibodies with different epitope specific to the TSH molecule. One of these antibodies is conjugated with biotin; the other is immobilised on the inner surface of wells. TSH molecules from the sample bind to both the immobilised antibody and anti-TSH-biotin conjugate. The wells are washed with wash buffer to remove material not bound on the inner surface of the wells, after which enzyme conjugate is added to each well. The wells are washed again to remove any conjugate not bound to the conjugated antibody and substrate is added to each well. The intensity of the colour that develops after the substrate incubation is proportional to the amount of antigen in the sample. Results of samples can be determined directly using the standard curve.

4. **WARNINGS AND PRECAUTIONS**
1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Make sure that everything is understood.
3. Should the kit package be severely damaged, please contact IBL or your supplier in writing, no later than one week after receiving the kit. Do not use damaged components in test runs. Instead, keep them safe for complaint-related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-website or directly from IBL on request.
7. Chemicals and reagents prepared or used must be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Professionals should guide the cleaning staff regarding potential hazards and handling.
9. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.
10. Avoid contact with Stop solution. It may cause irritations and burns.
5. STORAGE AND STABILITY
The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.
The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Blood Spots

| Blood from the newborn's heel should be collected only from the medial or lateral section of the plantar surface. Usual precautions for blood collection should be observed. After puncturing of the heel, the first drop of blood should be wiped away with a sterile gauze. |
| Touch the collection card against a large suspended drop of blood and allow a sufficient quantity of blood to soak into the filter paper in one go so that it fills the pre-printed circle completely. Repeat the procedure to fill the required number of pre-printed circles on the collection card. |
| Allow the blood spots to air-dry for 3 h at room temperature away from direct sunlight. |

⚠️ Because the standards are spotted on filter cards from Whatman 903® and the filter card material has a significant influence on the results (see LIMITATIONS OF THE PROCEDURE), Whatman 903® paper cards MUST be used for the patient samples.

⚠️ Don’t squeeze the puncture site during the collection since this will cause haemolysis or dilution of the blood with tissue fluid. Don’t apply successive drops of blood to the same pre-printed circles. Don’t touch or smear the blood spots.

⚠️ Ensure the blood spot samples are visually okay (e.g. no blood smears, no coagulates, no fingerprints on the spots).

⚠️ The optimal sample collection point is between 48 - 72 hours after birth. Samples should not be taken before 36 hours after birth and no later than 72 hours after birth [6].

National and country specific guidelines to sample collection point of time must be considered.

| Storage: | 2-8°C |
| Stability: | 6 mon |

Keep away from heat or direct sunlight.
7. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Quantity (RE55105)</th>
<th>Quantity (RE55109)</th>
<th>Symbol</th>
<th>Component</th>
</tr>
</thead>
</table>
| 5 x 12 x 8         | 25 x 12 x 8        | MTP     | Microtiter Plate  
|                    |                    |         | Break apart strips. Coated with antibodies against TSH. |
| 1 x 6 x 8          | 2 x 6 x 8          | CAL A-F | Standard A-F  
|                    |                    |         | Ready to use. 
|                    |                    |         | Contains: human blood, human TSH on Whatman 903® paper. 
|                    |                    |         | 8 Blood Spots / card. For exact concentrations see labels or QC certificate. |
| 1 x 3 x 8          | 2 x 3 x 8          | CONTROL 1-3 | Control 1-3  
|                    |                    |         | Ready to use. 
|                    |                    |         | Control 1: low 
|                    |                    |         | Control 2: middle 
|                    |                    |         | Control 3: high 
|                    |                    |         | Contains: human blood, human TSH on Whatman 903® paper. 
|                    |                    |         | 8 Blood Spots / card. For concentrations / acceptable ranges see labels or QC certificate. |
| 1 x 15 mL          | 4 x 15 mL          | ASSAYBUF CONC | Assay Buffer, Concentrate (20x)  
| 1 x 400 µL         | 5 x 400 µL         | BIOTIN-AB CONC | Biotin anti-TSH Ab, Concentrate (100x)  
|                    |                    |         | Contains: Biotin-anti-TSH antibody and ProClin300. |
| 1 x 600 µL         | 5 x 600 µL         | ENZCONJ CONC | Enzyme Conjugate, Concentrate (100x)  
|                    |                    |         | Contains: streptavidin conjugated to peroxidase and stabilizers. |
| 5 x 15 mL          | 4 x 90 mL          | TMB SUBS | TMB Substrate Solution  
|                    |                    |         | Ready to use. Contains: Buffer, TMB, stabilizers. |
| 5 x 15 mL          | 4 x 90 mL          | TMB STOP | TMB Stop Solution  
|                    |                    |         | Ready to use. Contains: 1 M H₂SO₄. |
| 2 x 100 mL         | 10 x 100 mL        | WASHBUF CONC | Wash Buffer, Concentrate (10x)  
|                    |                    |         | Contains: phosphate buffer, Tween. |
| 15 x 15 x          | FOIL               |         | Adhesive Foil  
|                    |                    |         | black |

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 50; 100-500; 1000 µL
2. Blood collection cards (Whatman 903®)
3. Blood spot puncher, 3 mm (e.g. Sauer, Hannover, Germany)
4. Microtiter plate shaker (300-500 rpm; amplitude 1.5-3.0 mm)
5. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
6. Empty vials for preparation of Biotin and Enzyme Conjugate
7. Vortex mixer
8. 8-Channel Micropipettor with reagent reservoirs
9. Wash bottle, automated or semi-automated microtiter plate washing system
10. Bidistilled or deionised water
11. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps must be followed strictly and in line with the instructions. Use calibrated pipettes and devices only.
2. Once the test has been initiated, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap vials that are not used. Do not reuse wells/tubes or reagents. Unused wells should be returned immediately to the resealed pouch including the desiccant.
4. It is advised to determine standards, controls and samples in duplicate in order to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. The following precautions should be taken when dried blood spots for the TSH determination are punched:
   1. Punch the discs only from uniformly-covered dried blood spots.
   2. For double determinations, use only two nearby discs from one blood spot only, in order to avoid a chromatographic effect as much as possible.
9. Ensure all dried blood spots, without exception, are removed from the microtiter plate before starting washing.
10. Check the blood spot samples are visually okay (e.g. no blood smears, no coagulates, no finger-prints on the spots).
11. Unused wells/tubes and dried blood spots should be returned immediately to the resealed pouch, including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

Preparation of concentrated components (1 Microtiter Plate)

<table>
<thead>
<tr>
<th>Dilute / dissolve</th>
<th>Component</th>
<th>with</th>
<th>Diluent</th>
<th>Relation</th>
<th>Remarks</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mL</td>
<td>WASHBUF CONC</td>
<td>900 mL bidist. water</td>
<td>1:10</td>
<td>Mix vigorously.</td>
<td>2-8°C</td>
<td>4 weeks</td>
<td></td>
</tr>
<tr>
<td>2 mL</td>
<td>ASSAYBUF CONC</td>
<td>38 mL bidist. water</td>
<td>1:20</td>
<td>Mix thoroughly.</td>
<td>2-8°C</td>
<td>2 weeks</td>
<td></td>
</tr>
<tr>
<td>60 µL</td>
<td>BIOTIN-AB CONC</td>
<td>6 mL diluted Assay Buffer</td>
<td>1:101</td>
<td>Mix &gt; 10 min without foaming.</td>
<td>RT</td>
<td>up to 3h</td>
<td></td>
</tr>
<tr>
<td>110 µL</td>
<td>ENZCONJ CONC</td>
<td>11 mL diluted Assay Buffer</td>
<td>1:101</td>
<td>Mix &gt; 10 min without foaming. Change of color: red → orange</td>
<td>RT</td>
<td>up to 3h</td>
<td></td>
</tr>
</tbody>
</table>

Allow kit to reach room temperature (18-25°C). Mix reagents before preparing the solutions. Precipitates that may be present in the assay buffer will dissolve when liquid reaches room temperature. If you are using several vials of the concentrated Enzyme Conjugate, it is highly recommended to pool the solutions and to establish the working solution from this pool. Do not use polypropylene bottles for the preparation of Enzyme Conjugate.
11. TEST PROCEDURE

1. Punch out one disc of each blood spot Standard (A - F), Control 1-3 and sample with a blood spot puncher and put the discs into the assigned well of the coated MTP.

2. Pipette 100 µL of diluted Assay Buffer into each well. Cover plate with black adhesive foil.

3. Incubate 60 min at RT (18-25°C) on a plate shaker (300–500 rpm; 1.5–3.0 mm amplitude). Make sure that all discs are immersed in diluted Assay Buffer.

4. Remove adhesive foil. Pipette 50 µL of diluted Biotin-AB into each well. Cover plate with black adhesive foil.

5. Incubate 60 min at RT (18-25°C) on a plate shaker (300–500 rpm; 1.5–3.0 mm amplitude).

6. Remove adhesive foil. Discard incubation solution and all discs by tapping the inverted plate on a paper towel. Wash plate 5x with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.

7. Pipette 100 µL of diluted Enzyme Conjugate into each well. Cover plate with black adhesive foil.

8. Incubate 60 min at RT (18-25°C) on a plate shaker (300–500 rpm; 1.5–3.0 mm amplitude).

9. Remove adhesive foil. Discard incubation solution. Wash plate 5x with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.

10. If available, use an 8-Channel Micropipettor for adding Substrate and Stop Solution. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.

11. Pipette 100 µL of TMB Substrate Solution into each well.

12. Incubate 30 min at RT (18-25°C) on a plate shaker (300–500 rpm; 1.5–3.0 mm amplitude).

13. Stop the substrate reaction by adding 100 µL of TMB Stop Solution into each well. Briefly mix contents by gently shaking the plate.

14. Measure optical density with a photometer at 450 nm (reference wavelength: 600-650 nm) within 15 min after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed according to the instructions. Moreover the user must adhere strictly to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system for obtaining diagnosis according to GLP. All kit controls must be within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. Participating in appropriate quality assessment trials is recommended.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.
13. CALCULATION OF RESULTS

13.1. Determining the standard curve

The obtained OD of the standards (y-axis, linear) is plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with linear regression or 4 Parameter Logistics.

To calculate the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

**Standards are calibrated according to 3rd ISNS Reference Preparation for Neonatal Screening.**

The results are expressed in mIU/L whole blood. To convert them into mIU/L serum, please multiply the obtained results by two.

**Typical Calibration Curve**

(Example: Do not use for calculation!)

<table>
<thead>
<tr>
<th>Standard</th>
<th>mIU/L</th>
<th>ODmean</th>
<th>OD/ODmax (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>0.094</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>0.235</td>
<td>11</td>
</tr>
<tr>
<td>C</td>
<td>29</td>
<td>0.634</td>
<td>29</td>
</tr>
<tr>
<td>D</td>
<td>57</td>
<td>1.153</td>
<td>53</td>
</tr>
<tr>
<td>E</td>
<td>88</td>
<td>1.707</td>
<td>78</td>
</tr>
<tr>
<td>F</td>
<td>112</td>
<td>2.176</td>
<td>100</td>
</tr>
</tbody>
</table>

14. EXPECTED VALUES

Various societies for neonatal screening recommend different cut-off values for repetition of the measurement and the application of confirmatory assays. Depending on the application of samples of different populations of newborns it is highly recommended that each laboratory establishes its own range of normal values and that this distribution of values is co-ordinated with the recommendations of the responsible society of this geographic region.

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

The following table can be used as an indication for result interpretation. These ranges refer to other commercially available assays to which the results received with the IBL TSH neonatal screening ELISA correlate well and which are calibrated against the same reference material. Furthermore there is evidence from scientific literature to interpret the results as follows:

<table>
<thead>
<tr>
<th>TSH</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10 mIU/L</td>
<td>normal</td>
</tr>
<tr>
<td>10 – 20 mIU/L</td>
<td>equivocal</td>
</tr>
<tr>
<td>&gt; 20 mIU/L</td>
<td>hypothyroid</td>
</tr>
</tbody>
</table>

15. LIMITATIONS OF THE PROCEDURE

Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

Any result with an elevated concentration has to be indicated as 'presumed positive' and has to be confirmed with further sampling and testing. A false negative result of this assay cannot be excluded with absolute certainty.

Conditions which are known to cause anomalous analytical assay results include:
- sample spot not uniformly saturated with blood
- sample spots punched too close to the edge of the blood spot
- poorly collected and improperly dried specimens
- non-eluting blood spot due to deterioration of sample caused by exposure to heat and humidity
- contamination of blood spot filter paper with faecal material
For cross-reactivities, see PERFORMANCE.

The following blood components do not have a significant effect on the test results up to the concentrations stated below:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>5 mg/mL</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>91 mg/mL</td>
</tr>
</tbody>
</table>

16. PERFORMANCE

<table>
<thead>
<tr>
<th>Analytical Specificity (Cross Reactivity)</th>
<th>No crossreactivity was detected for the following substances, tested up to 10 times their normal concentration in blood: hLH, hFSH, hCG, T3 and T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision</td>
<td>Range (mIU/L)</td>
</tr>
<tr>
<td>Intra-Assay</td>
<td>7.0-50.1</td>
</tr>
<tr>
<td>Inter-Assay</td>
<td>8.8-83.8</td>
</tr>
<tr>
<td>Inter-Lot</td>
<td>8.2-82.6</td>
</tr>
</tbody>
</table>

Method Comparison versus Other Assay

<table>
<thead>
<tr>
<th>IBL-Assay</th>
<th>IBD-Assay = 0.96 x CDC / UKNEQAS Samples + 1.94</th>
<th>IBD-Assay = 0.73 x commercial kit 1 - 0.50</th>
<th>IBD-Assay = 1.00 x commercial kit 2 - 0.31</th>
<th>IBD-Assay = 1.37 x commercial kit 3 - 0.97</th>
<th>IBD-Assay = 0.85 x commercial kit 4 - 0.97</th>
</tr>
</thead>
<tbody>
<tr>
<td>r = 0.98; n = 37</td>
<td>r = 0.98; n = 37</td>
<td>r = 0.98; n = 37</td>
<td>r = 0.97; n = 35</td>
<td>r = 0.94; n = 37</td>
<td></td>
</tr>
</tbody>
</table>

17. PRODUCT LITERATURE REFERENCES

## Symbols / Symbole / Symbôles / Símbolos / Σύμβολα

<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:</td>
</tr>
<tr>
<td></td>
<td>Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:</td>
</tr>
<tr>
<td>CONC</td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συµπύκνωµα</td>
</tr>
<tr>
<td>LYO</td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Λυοφιλιασµένο</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.</td>
</tr>
<tr>
<td></td>
<td>Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell’uso. / Διαβάστε τις οδηγίες πριν την χρήση.</td>
</tr>
<tr>
<td></td>
<td>Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερµότητα και άµεση επαφή µε το φως του ηλίου.</td>
</tr>
<tr>
<td></td>
<td>Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:</td>
</tr>
<tr>
<td></td>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:</td>
</tr>
<tr>
<td></td>
<td>Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!</td>
</tr>
</tbody>
</table>

Symbols of the kit components see MATERIALS SUPPLIED.  
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.  
Voir MATERIEL FOURNI pour les symbôles des composants du kit.  
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.  
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.  
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.  
Για τα σύµβολα των συστατικών του κιτ συµβουλεύετε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.