Neonatal TSH ELISA KIT

There are 96 (200) determinations permitting the construction of one standard curve and the assay of 40 unknowns in duplicate.

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 triiodothyronine (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 by 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.

Principle of method
The technology uses two high affinity monoclonal antibodies in an immunometric assay system. The two antibodies react simultaneously with the antigen present in standards or samples. This reaction leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich". In the standard solid-phase ELISA system the reaction is carried out in a microtiter plate (2 strips) which acts as the binder of sandwich complex.

In the present product blood standards and samples collected on filter paper are incubated with the conjugate which contains the horse radish peroxidase (HRPO) labelled antibody at room temperature for 2 hours. After washing the microplate to remove the filter paper and unbound components of the sample and the conjugate, a ready-to-use tetramethylbenzidine (TMB)/peroxide substrate is added to each well and incubated. The signal is measured in an ELISA photometer at 450 nm wavelength. (620 nm as reference wavelength is recommended)

The concentration of TSH is directly proportional to the optical density measured in the wells. The unknown concentration of TSH in patient samples is read off a calibration curve constructed by plotting binding values against a series of calibrators containing known amount of TSH.

Contents of the kit
96 wells pack size, EK-1N

1. 1 vial CONJUGATE (22 ml), ready to use, containing anti-hTSH antibodies in buffer with blue dye and 0,01 % merthiolate and 0,2% chloracetic acid (CBA).
2. 2 pieces of TSH STANDARD SETS, 6 levels / set. Nominal concentrations are 1, 10, 20, 40, 80 and 160 mIU/l TSH in whole human blood, prepared on filter paper S&S 903 and calibrated against the WHO 2nd IRP 80/558.
3. 2 pieces of CONTROL CARDS, two levels / card. About 17 and 45 mIU/l TSH in whole human blood prepared on filter paper S&S 903. The concentrations of the controls are specified in the quality certificate enclosed.
4. 1 vial DILUENT (5,5 ml), horse serum to eliminate the non specific binding, with 0,1% sodium azide.
5. 1 vial SUBSTRATE (25 ml) ready to use, in brown plastic bottle. TMB. Do not expose to direct light!
6. 1 piece MICROTITER PLATE, ready to use, 12 strips, packed in an air-tight foil.
7. 1 bottle WASH BUFFER CONCENTRATE (20 ml) with 0,01 % merthiolate.

Preparation of reagents.
8. 1 vial STOP REAGENT (6 ml), 1M sulfuric acid
   Cover stick
   Plate map
   Quality certificate
   Pack leaflet

960 wells pack size, EK-1N10

1. 2 bottles CONJUGATE (2 x 110 ml).
2. 5 pieces of TSH STANDARD SETS, 6 levels / set.
3. 5 pieces of CONTROL CARDS, two levels / card.
4. 1 bottle DILUENT (55 ml), horse serum
5. 1 bottle SUBSTRATE (250 ml) ready to use.
6. 10 pieces MICROTITER PLATE, ready to use.
7. 1 bottle WASH BUFFER CONCENTRATE (200 ml) with 0,01 % merthiolate.

Preparation of reagents.
8. 1 bottle STOP REAGENT (60 ml), 1M sulfuric acid
   Cover stick, 20 pcs
   Plate map, 10 pcs
   Quality certificate
   Pack leaflet

1920 wells pack size, EK-1N20

1. 4 vials CONJUGATE (4 x 110 ml),
2. 10 pieces of TSH STANDARD SETS, 6 levels / set.
3. 10 pieces of CONTROL CARDS, two levels / card.
4. 1 bottle DILUENT (110 ml), horse serum
5. 1 bottle SUBSTRATE (500 ml) ready to use.
6. 20 pieces MICROTITER PLATE, ready to use.
7. 2 bottles WASH BUFFER CONCENTRATE (2 x 200 ml) with 0,01 % merthiolate.

Specimen collection and storage
Collect a blood sample from the heel of the infant as follows. Clean the heel of the infant with soap and water. Wipe area dry. Use alcohol on the area. Air dry.
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 been formed use a sample collection card to collect the infant’s blood on the card. Do not tear the filter paper surface. To avoid haemolysis and dilution of the blood sample do not exert excessive pressure during collection.
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. Within 24 hours, place each sample in its own individual paper envelope. Place in a moistureproof bag at 2-8°C for short-term storage and -20°C for long-term storage.

Preparation of reagents, storage
Except the wash buffer concentrate each reagent is supplied in ready to use form.

96 well size - Add the wash buffer concentrate (20 ml) to 600 ml distilled water to obtain 620 ml wash solution.
960 and 1920 well size - Add the wash buffer concentrate (200 ml) to 6 l distilled water to obtain 6.2 litre wash solution.
Upon dilution store at 2-8°C until expiry date. Store the rest of reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date. The actual expiry date is given on the package label and in the quality certificate.
Assay procedure

(For a quick guide, refer to Table 1.)

1. Equilibrate reagents to room temperature before use. Homogenize all reagents by gentle mixing to avoid foaming.
2. Label the plate map for duplicates of each standard (S1-S6), controls (C1-C2) and samples (Mx).
3. Dispense standards, controls and samples by punching out 3 mm blood spotted filter paper into the appropriate wells.
4. Pipette 200 μl of conjugate solution into each well.
5. Pipette 50 μl of diluent into each well.
6. Cover the plate by the enclosed foil band and place the plate immediately on an absorbent tissue and blot dry by hitting plate. Make sure that all of the spots have been removed.
7. Add 300 μl wash buffer into each well, then decant (tap and blot) or aspirate. Repeat this step 5 times. An automatic or manual plate washer can be used. Follow manufacturer’s instruction for proper usage.
8. Pour the incubation mixture (including blood spotted filter paper) by flicking the plate contents into the waste container. Holding in the upside down position place the plate immediately on an absorbent tissue and blot dry by hitting plate. Make sure that all of the spots have been removed.
9. Add 500 μl wash buffer into each well, then decant (tap and blot) or aspirate. Repeat this step 5 times. An automatic or manual plate washer can be used. Follow manufacturer’s instruction for proper usage.
10. Pour the substrate into its plastic tray, and pipette 200 μl to each well with the aid of the multi-channel pipette. Place the plate into the dark for 30 minutes. If less than the whole volume is used in one assay, do not pipette directly from the bottle, and never fill the unused reagent back into its original bottle.
11. Pipette 50 μl stop reagent into each well, and shake gently for 30 seconds.
12. Measure in the ELISA photometer at 450nm within 20 minutes after adding the stop solution. (620 or 630 nm as second wavelength is recommended)
13. Calculate the concentrations of the samples as described in Calculation of results.

Calculation of results

The calculation is illustrated using representative data. Data obtained should be similar to those shown in Table 2.

Manual calculation

Calculate the average OD for each pair of duplicates. Draw the standard curve on a linear graph paper by plotting calculated OD of each standard level (ordinate) against the respective concentration (abscissa). Obtain sample values by interpolation of sample OD values on the standard curve.

Data evaluation using normalized binding

For computerised calculations and/or quality assessment normalised specific binding values, rather than OD values are used. Specific binding values can be calculated for each standard and sample according to the following equation:

\[ \frac{S1 - S6}{C1 - C2} \times \frac{S6}{OD} \times 100 \]

Table 2. Typical assay data

<table>
<thead>
<tr>
<th>OD</th>
<th>OD mean</th>
<th>B/Bmax, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.0971</td>
<td>0.0960</td>
</tr>
<tr>
<td>S2</td>
<td>0.0493</td>
<td>0.2149</td>
</tr>
<tr>
<td>S3</td>
<td>0.4512</td>
<td>0.4503</td>
</tr>
<tr>
<td>S4</td>
<td>0.8432</td>
<td>0.8089</td>
</tr>
<tr>
<td>S5</td>
<td>1.4305</td>
<td>1.3975</td>
</tr>
<tr>
<td>S6</td>
<td>2.6492</td>
<td>2.6893</td>
</tr>
</tbody>
</table>

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

<table>
<thead>
<tr>
<th>wells</th>
<th>Standard</th>
<th>Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>3 mm filter paper punch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>200 200 200</td>
<td>50 50 50</td>
<td>50 50 50</td>
</tr>
<tr>
<td>Conjugate</td>
<td>200 200 200</td>
<td>50 50 50</td>
<td></td>
</tr>
<tr>
<td>Diluent</td>
<td>50 50 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shake for 2 hours at room temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decant the fluid and spots. Blot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash buffer</td>
<td>300 300 300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decant the fluid and blot on filter paper</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeat the washing step 5 times</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substrate</td>
<td>200 200 200</td>
<td>50 50 50</td>
<td></td>
</tr>
<tr>
<td>30 minutes at room temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop reagent</td>
<td>50 50 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measurement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculate the results</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Characterization of assay

Typical assay parameters

B/ODmax < 5 %

Sensitivity

The minimal detectable concentration of TSH was determined by adding 2 SD to the mean optical density value of 15 standard-1 (1 mIU/l) replicates and calculating the corresponding concentration from the standard curve. The minimum difference perceptible from the first standard is 0.95 mIU/l.

Figure 1: A typical standard curve (Do not use to calculate unknown samples!)

Hook effect

There is no high dose “hook effect” up to the TSH concentration of 30000 mIU/l.

Specificity

This kit exhibits no significant detectable cross-reactivity with HCG, hLH, hFSH.

Precision

2 patient samples were assayed in 16 replicates to determine intra-assay precision.

Reproducibility

To determine inter-assay precision two patient samples were measured in duplicates in 10-15 independent assays by 2 operators using different kit batches. Values obtained are shown below.

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking blood samples with known amount of TSH. 89-108% was obtained for 5 whole blood samples.

Expected Values

Neonates 0-3 days: less than 25 mIU/l.

Procedural notes

A thorough understanding of this package insert is necessary for successful use of the kit. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.

To avoid contamination of samples and standards use clean tweezers when handling, not hands.

When punching the provided standard calibrator card, make sure that the provided standards are punched from low to high concentration.

Always add reagents in the same order to minimize reaction time differences between wells.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
Avoid microbial contamination of reagents, especially of the Conjugate solution and TMB substrate:

- Do not leave the cap off of the storage bottle for prolonged periods of time.
- Never pipette directly from the bottle. Always pour just the necessary volume into a separate container for use then discard the excess after use.

The results should be read within 15 minutes of adding the Stop solution.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precaution

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1) and Hepatitis B surface Antigen (HBsAg).

Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood samples should therefore be handled as potentially infectious materials.

Chemical hazard

Components contain sodium azide as antimicrobial agents. The total azide present in each pack is 5.5 mg. (96 wells pack size)

Caution! The stop reagent is corrosive. Avoid contact with it because may cause skin irritations and burns.

Website: http://www.izotop.hu