



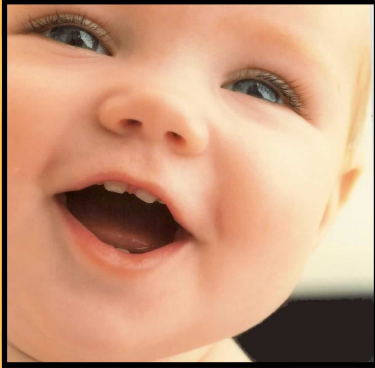
Neonatal Product Catalog

- Quantitative Test
- Non-radioactive Test
- Easy Procedure
- High Sensitivity
- High Specificity
- Simple Equipments

GBI TSH NEONATAL SCREENING KIT

An Enzyme Immunoassay (EIA) for the quantitative Determination of Thyroid Stimulating Hormone (TSH) Levels in Neonates

GBI TSH NEONATAL SCREENING KIT
(Cat. No. NTSH576)



**GBI TSH Neonatal
Screening Kit Components**

Catalog No.: NTSH576
Tests per kit: 576 tests

Components:

1. TSH Capture Microplate:
6 x 96 wells
2. TSH Elution Buffer:
1 x 70ml
3. Anti-TSH PO Conjugate
1 x 1.4ml
4. TSH Diluent Buffer
1 x 70ml
5. PO Wash Buffer (20x)
1 x 100ml
6. PO Color Reagent
1 x 70ml
7. PO Color Stopper
1 x 70ml
8. TSH Dried Blood Standards
and Controls
1 set

GBI TSH NEONATAL SCREENING KIT
(Catalog No.: NTSH576)

- Elisa Quantitative Test
- No radioactive material contamination
- Screened more than 6 millions babies
- High Sensitivity
- High Specificity
- Simple Equipments

Background & Intended Use

Background:

Congenital hypothyroidism (CH) is one of the most common metabolic disorders resulting in permanent mental retardation if undetected or left untreated soon after birth. Newborns that have been identified and treated for CH within two weeks of birth can be expected to have normal cognitive development. Screening for Congenital Hypothyroidism (CH) is important for the prevention of irreversible mental retardation. Determination of TSH in neonatal blood dried on filter paper (the Guthrie Card) is used as a primary screening test.

Intended Use:

For the quantitative determination of TSH (thyroid stimulating hormone, thyrotropin) in blood dried on filter paper.

Screened more than 6,000,000 babies !

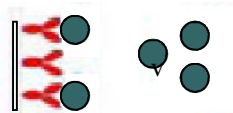
Principle of Assay

The GBI TSH Neonatal Screening Kit is an enzyme immunoassay. A highly specific anti-hTSH (human) antibody has been immobilized onto each well of the 96-well microplates provided. To begin the assay, sample discs punched from dried whole blood spot standards, controls and neonate specimens are added to the coated wells. An elution buffer is also added. The plate is incubated to elute TSH from the sample disc and to allow capture of the eluted TSH by the antibody immobilized onto the microplate wells. Following incubation the plates are washed to remove the sample discs as well as the eluate.

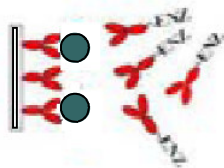
A second antibody, a β -specific anti-hTSH monoclonal that has been conjugated to the enzyme horseradish-peroxidase (HRP), is then added to the wells and incubated. The eluted TSH of the sample already captured by the microplate-bound antibody is now also bound by the enzyme-conjugated monoclonal antibody added. An antibody-TSH-antibody bridge, or "sandwich", forms that is bound to the surface of the microplate wells. Any unbound complexes are removed with subsequent plate washings.

The final stage of the assay is the detection of the microwell-bound complexes by the addition of a color developing reagent. The enzyme (HRP) portion of the bound "sandwich" reacts with the color developer, 3, 3', 5, 5'-Tetramethylbenzidine (TMB) in the presence of hydrogen peroxide (H_2O_2). The TMB/ H_2O_2 liquid is converted from colorless to blue. The degree of color change is directly proportional to the amount of TSH antigen that is bound in the well. The color development is terminated with the addition of a color stopper that converts the blue to yellow.

The results are measured with a microplate reader at a wavelength of 450 nm. The absorbance measured is directly proportional to the concentration of TSH in the sample. A standard curve is generated by plotting the light absorbance of each standard versus its known TSH concentration. The concentrations of TSH in the unknown samples are determined by interpolation from this standard curve.



1. Antigen is captured by antibody pre-coated on solid phase during elution incubation. Unbound antigen is washed away.



2. Conjugated antibody directed against antigen are added to the solid phase.



3. Binding of the conjugated antibody form the "Sandwich". Unbound conjugated antibody is washed away.



4. Chromogen/substrate is added and color is allowed to developed for a defined time. The reaction is stopped by Color Stopper reagent and the color is quantified by a spectrophotometer.



Blood Collection Procedures

1. Prepare sterile lancet with tip 2mm, sterile alcohol prep, gauze pad, blood collection form, and gloves.
2. Complete all information on the collection form. DO NOT contaminate filter paper circle.
3. Warm the puncture site up to 40°C for 3-5 minutes
4. Cleanse site with alcohol prep. Wipe dry with sterile gauze pad.
5. Puncture heel. Make sure to puncture the safe site on the foot heel.
6. Wipe away the first blood drop with gauze pad. Allow another LARGE drop to form.
7. Lightly touch the filter paper to the LARGE blood drop. Allow blood to soak thoroughly and completely to file the circle. Apply blood on one side of the filter paper.
8. Fill the remaining circles with the same manner as step
9. Apply care of puncture site.
10. Place blood spot on a dry, clean area for 4 hours and mail it to a testing laboratory.

GBI TSH NEONATAL SCREENING KIT
(Cat. No. NTS576)



Assay Procedures

As simple as 5 steps:

- 1. Elute and incubate specimen over night**

Wash



- 2. Incubate antibody-enzyme conjugate for 2-3 hours**

Wash



- 3. Add Color Reagent for 30 minutes**

Wash



- 4. Add Color Stopper**

- 5. Measure the absorbance of each well**

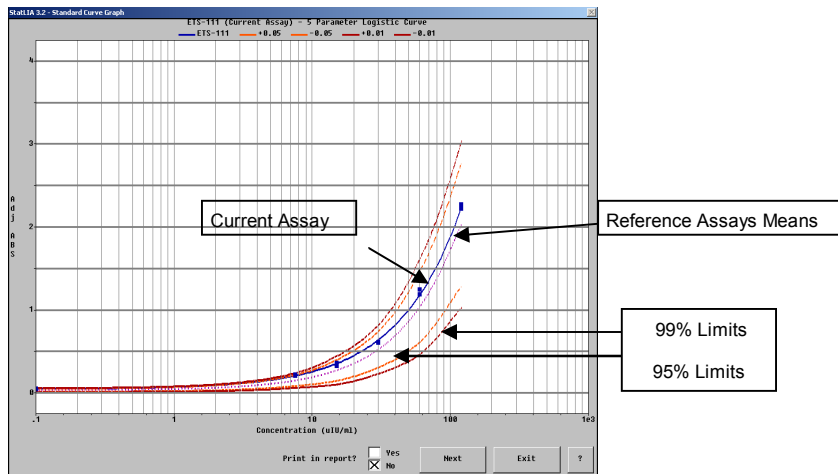
Assay Procedures

1. Create a plate map designating the well positions of all standards, controls and neonate samples to be assayed.
2. Punch a 3mm (1/8 inch) diameter sample disc from each of the standard, control and neonate samples and place it into its designated well (s).
3. Pipette 100µl of Elution Capture Buffer (TEC) into each well containing a sample disc. Cover the plate with sealing tape (provided). Incubate the microplate while rotating to mix (approx. 100 rpm). Incubate overnight (16-24 hrs) at room temperature (18-25° C).
4. At the end of the incubation period carefully remove the sealing tape and remove the sample discs to waste. Wash the plate four (4) times with a minimum of 350µl per well of PO Wash Buffer Solution for each wash.
5. Add 100µl of freshly prepared Antibody-Enzyme Conjugate Solution to each well. Cover the microplate with (new) sealing tape and rotate to mix at room temperature (18-25° C) for approx. 2-3 hours.
6. At the end of the incubation period carefully remove the sealing tape and wash the plate four (4) times.
7. Add 100 µl of PO Color Reagent to each well. Cover the microplate with a re-usable plastic lid (do not use sealing tape) and rotate to mix at room temperature (18-25° C) for 30 minutes.
8. At the end of the incubation period add 100 µl of PO Color Stopper to each well.
9. Measure the light absorbance of each well using a microplate reader at a wavelength setting of 450 nm. Measure within 15 minutes of adding the PO Color Stopper.
10. Prepare a standard curve by plotting the known concentration of each standard on the x axis and the corresponding absorbance on the y axis. Unknown concentrations are determined by comparison with this standard curve.

Screened more than 6,000,000 babies !

Result Calculation

An example of A representative standard curve is provided below. This example was plotted using a 5 parameter logistic curve fit. The mean and confidence limits are displayed showing the 95% and 99% probability of absorbance response from a group of previously performed assays.



Avoid Common Errors of Invalid Specimen

1. Insufficient specimen for testing because of removing filter paper before blood has completely filled the circle.
2. Specimen appear scratched by capillary tube or other device.
3. Mailing specimen out before drying.
4. Applying excess blood to filter paper.
5. Squeezing or “milking” of area surrounding the puncture site.
6. Specimen appear diluted or discolored due to contamination of alcohol, gloves, hands or antiseptic solution or exposing blood spots to direct heat.
7. Specimen exhibits serum ring because of not wiping alcohol from puncture site before making skin puncture or wrong drying procedure.
8. Specimen appear clotted or layered due to touching the same circle on filter paper to blood drop several times.

Expected Values and Interpretation of Results

A variety of factors will determine the normal range for neonatal TSH concentrations. Demographic variations, the age and weight of the infant at sample collection, multiple births and infants born prematurely are all factors which can affect the cut-off values for normal concentrations of TSH in a neonatal screening program. For these reasons each laboratory should establish normal ranges and cutoff values for their individual application.

GBI TSH EIA	TSH Result (uIU/ml serum equivalent)		
	95th Percentile	97.5th Percentile	99th Percentile
N = 980	15.2	17.3	20.4

Table 1: GBI Expected Values at the 95th, 97.5th and 99th Percentiles for a Population of Presumed Normal Neonates; N=980*

* Percentile values were obtained per NCCLS C28-A2, using the MS Excel “Rank and Percentile” data analysis tool.

The values shown here were calculated based on a specific population of samples as defined by the manufacturer. Each laboratory should determine expected values for normal TSH concentrations within its own screening population. Recommendations published by the American Academy of Pediatrics and the American Thyroid Association Committee on Public Health² for the interpretation of screening results can be summarized as:

<u>TSH uIU/ml Serum Equivalent</u>	<u>Interpretation</u>
< 20	Normal
20 – 40	Borderline
> 40	Hypothyroid

GBI TSH NEONATAL SCREENING KIT (Cat. No. NTSH576)



FAQ

Q: Has the GBI TSH Neonatal Screening Kit been tested in screening laboratories?

A: GBI TSH Neonatal Screening Kit has been used in many newborn screening laboratories in China and Greater Asia. More than **six million** newborns have been screened using our kit.

Q: Has the GBI TSH Neonatal Screening Kit been approved by FDA or other international standards?

A: GBI TSH Neonatal Screening have been approved by **FDA 510K** standards.

Q: What laboratory equipments are required to use the GBI TSH Neonatal Screening Kit? Can the kit be run in automatic station and manual?

A: The kit can be run with automatic liquid handling station. It also can be run manually. Simple equipments such as multi-channel pipettes and microplate reader capable of reading at a wavelength of 450 nm are needed.

Assay Performance Characteristics

1. Precision:

Precision studies were conducted in accordance with NCCLS EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition. Testing included two sample aliquots per run, one run per day, performed on 20 different days using the GBI published assay procedure. The resulting data were used to estimate repeatability, between-day and within-device precision as described by the standard. Sources of variability included the use of different operators, days and reagent lot numbers.

Estimates of Precision for GBI TSH EIA Kit:

Within-Run Standard Deviation :	2.6
Between-Day Standard Deviation :	2.6
Within Device Standard Deviation:	3.7

2. Analytical Sensitivity:

The Analytical Limit at Low Levels (limit of sensitivity) for the GBI TSH EIA Kit was determined by testing the zero standard multiple times (N=20) within a single assay. The resulting data were used to calculate the analytical limit at low levels. Refer to Table 2, below. The analytical sensitivity is defined as the calculated concentration that corresponds to the mean of the absorbance values of the zero standard (N=20) plus two times the standard deviation derived from those same (N=20) absorbance values. These data are provided for example only. Each laboratory should establish appropriate working limits based upon their own patient population and/or data.

Table 2: Summary Results and Analytical Limits at Low Levels for GBI TSH EIA

	GBI TSH
Count	20
Mean ABS	0.0354
SD	0.0063
%CV	18
Min	0.0289
Max	0.0570
Mean + (2 *S.D.) ABS:	0.0480
Analytical Limit (uIU/ml)	2.4

3. Linearity, Assay Measurable (Reportable) Range:

Linear Regression Analysis:
 $y = 0.8997x + 0.6036$, $R^2 = 0.999$

Average Recovery-Overall %: 95.2

Screened more than 6,000,000 babies !

Assay Performance Characteristics

4. Specificity:

The following compounds were tested for cross-reactivity. Each compound was added at several concentrations to TSH-free whole blood which had been adjusted to a hematocrit of 55%. The samples were spotted onto Whatman 903® specimen collection paper, air-dried and assayed. Refer to Table 3, below.

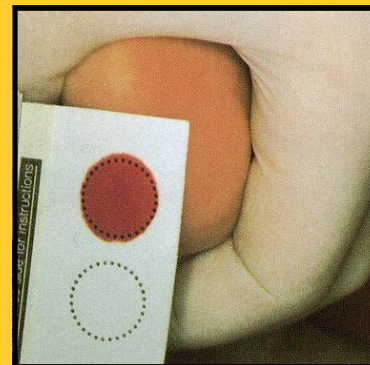
Table 3: Results of a Cross-Reactivity Study of the GBI TSH EIA Screening Assay

Peptide Hormone	Cross-Reactant Concentration Added (µIU/ml)	TSH Concentration Measured (µIU/ml)
FSH (WHO 2nd IRP HMG)	125	None Detected*
	250	None Detected*
	500	None Detected*
LH (WHO 1st IRP 68/40)	125	None Detected*
	250	None Detected*
	500	None Detected*
HCG (WHO 2nd I.S. 61/6)	10,000	None Detected*
	50,000	None Detected*
	100,000	None Detected*

* "None Detected" represents values obtained that were below the limit of detection for the assay (i.e., < 2.4 µIU/ml.)

Interference Studies

No interference with expected values of any clinical significance was observed from bilirubin and hemoglobin at any concentration.



Good Punching Techniques on Collection Card

1. Bring the Collection Card to room temperature before removing them from sealed plastic bags to prevent moisture caused by sudden temperature change.
2. Wear gloves and never touch the blood spots with bare hand.
3. Clean the puncher regularly. Avoid getting punch dust onto your body.
4. Always punch from the opposite side of blood application to obtain sufficient blood spot on both sides of the filter paper.
5. Avoid punching the edge of the blood spots.
6. Avoid static problems with room humidifiers.

Assay Tips

1. How to extract blood completely from the dried blood spot?

Extracting blood completely from the dried blood spot on filter paper is critical. Incomplete extraction will result in insufficient specimen that may lead to false negative cases. Overnight elution time helps to extract blood from specimen that experienced longer transportation period. Plates need to be sealed with tape and covered with lids during the entire elution period. When blood is completely extracted the color of blood spot on the filter paper will fade and the color of the elution buffer in each well will turn red.

2. How to reduce background or "edge effect"?

Evaporation is the main cause of background or edge effect. Since such evaporation is more pronounced in the outer wells resulting in what is called an "edge effect", the best technique to prevent "edge effect" is to seal a tape on each microplate and put a lid on the top plate. By placing the microplates in a Styrofoam box when rocking on a nutator one can achieve the best constant temperature distribution while keeping moisture in each well.

3. How to prepare a standard curve?

A standard curve is prepared by plotting the known concentration of each standard on the X axis and the corresponding absorbance on the Y axis. It is recommended to use at least a 5 parameter logistic curve to accurately calculate the value of TSH from the sample. It is not recommended to use a square quadratic curve or a linear curve.

4. How to optimize the incubation time?

Enzyme incubation time is directly affected by the temperature of a laboratory. Standard room temperature (20-25°C) provides a satisfactory environment for enzyme activity. Placing the enzyme conjugate reagent in room temperature for 30 minutes before assaying and keeping the temperature constant during the assay is very important to achieve optimal result.

How to Order

Information required for all orders

1. Order person and institution names
2. Phone, fax, or e-mail address
3. Purchase order number
4. Shipping and billing address
5. Credit card information
6. Catalog number, description and quantity
7. Special shipping instructions

Ordering

Place your order by e-mail, phone, FAX or On-line:

Phone: (425) 493-1801
Fax: (425) 493-1803
Web Site: www.gbi-inc.com
E-mail: info@gbi-inc.com

Customer Service hours: 9:00 a.m — 5:00 p.m
(Pacific Std.Time from Monday to Friday)

Shipping

All shipment are F.O.B. Concord, State of Washington, USA.
Freight and handling charges will be added to the invoice.

Returns

A return authorization and return number is required for all returns. Returned products due to order error will be charged 20% restocking fee. The 20% restocking fee only applies to un-opened, properly stored items. Freight must be paid by the buyer.

Term of Sale

Term of payment are net thirty (30) days from the invoice date upon credit approval by Golden Bridge International, Inc. A 1.5% service charge per month is added for accounts over 30 days. All sales subject to Golden Bridge International's standard practice and policies. We accept on-line order and credit card payment.

Returns

A return authorization and return number is required for all returns. Returned products due to order error will be charged 20% restocking fee. The 20% restocking fee only applies to un-opened, properly stored items. Freight must be paid by the buyer.

Conditions:

1. We reserve the rights to change prices or packaging at any time without prior notification
2. Golden Bridge International's products may not be re-sold, modified without approval by Golden Bridge International, Inc.
3. We guarantee that the performance of our products meets our stated claim. No guarantee of performance is made for unclaimed usage. We will replace or credit to products that do not meet Golden Bridge International's stated claims.

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