CHIRON | DIAGNOSTICS

ACS:180 Automated Chemiluminescence System

TSH +B

Contents

Catalog Number	Contents	Number of Tests
672220	6 vials of ACS:180 TSH Lite Reagent 6 vials of ACS:180 TSH Solid Phase ACS:180 TSH Master Curve card	300
or		
672221	1 vial of ACS:180 TSH Lite Reagent 1 vial of ACS:180 TSH Solid Phase ACS:180 TSH Master Curve card	50

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Intended Use

For the quantitative determination of thyroid-stimulating hormone (TSH, thyrotropin) in serum using the Chiron Diagnostics ACS:180® Automated Chemiluminescence Systems.

For In Vitro Diagnostic Use.

Materials Required But Not Provided

Catalog Number	Description	Contents
672181	Calibrator B	6 vials of low calibrator 6 vials of high calibrator
or		
672171	Calibrator B	2 vials of low calibrator 2 vials of high calibrator
672013	Septum Caps	100/package
672191	Wash Reagent 1	100 mL/vial

Optional Reagents

Catalog Number	Description
672177	Multi-Diluent 1 (50 mL/vial)
672422	TSH Master Curve Material
976000	Ligand 1, 2, 3

Summary and Explanation of the Test

Thyroid-stimulating hormone is a glycoprotein with two non-covalently bound subunits. The alpha subunit is similar to those of follicle-stimulating hormone (FSH), human chorionic gonadotropin (hCG), and luteinizing hormone (LH).[1-4]

The beta subunit of TSH is unique, which results in the specific biochemical and immunological properties of this hormone.

TSH is synthesized and secreted by the anterior pituitary in response to a negative feedback mechanism involving concentrations of FT_3 (free T_3) and FT_4 (free T_4). Additionally, the hypothalamic tripeptide, thyrotropin-releasing hormone (TRH), directly stimulates TSH production.

TSH interacts with specific cell receptors on the thyroid cell surface and exerts two main actions. The first action is to stimulate cell reproduction and hypertrophy. Secondly, TSH stimulates the thyroid gland to synthesize and secrete T_3 and T_4.

The ability to quantitate circulating levels of TSH is important in evaluating thyroid function. It is especially useful in the differential diagnosis of primary (thyroid) from secondary (pituitary) and tertiary (hypothalamus) hypothyroidism. In primary hypothyroidism, TSH levels are significantly elevated, while in secondary and tertiary hypothyroidism, TSH levels are low.

TRH stimulation differentiates secondary and tertiary hypothyroidism by observing the change in patient TSH levels. Typically, the TSH response to TRH stimulation is absent in cases of secondary hypothyroidism, and normal to exaggerated in tertiary hypothyroidism.

Historically, TRH stimulation has been used to confirm primary hyperthyroidism, indicated by elevated T_3 and T_4 levels and low or undetectable TSH levels. TSH assays with increased sensitivity and specificity provide a primary diagnostic tool to differentiate hyperthyroid from euthyroid patients.

Assay Principle

The Chiron Diagnostics ACS:180 TSH assay is a two-site sandwich immunoassay using direct, chemiluminometric technology, which uses constant amounts of two antibodies. The first antibody, in the Lite Reagent, is a monoclonal mouse anti-TSH antibody labeled with acridinium ester. The second antibody, in the Solid Phase, is a polyclonal sheep anti-TSH antibody which is covalently coupled to paramagnetic particles.

The system automatically performs the following steps:

- washes the sample probe with 200 µL of Wash Reagent 1
- dispenses 200 µL of sample into a cuvette
- dispenses 50 µL of Lite Reagent and 225 µL of Solid Phase and incubates for 7.5 minutes at 37°C
- separates, aspirates, and washes the cuvettes with reagent water[5]
- dispenses 300 µL each of Reagent 1 and Reagent 2 to initiate the chemiluminescent reaction
- reports results according to the selected option, as described in the system operating instructions or in the online help system

A direct relationship exists between the amount of TSH present in the patient sample and the amount of relative light units (RLUs) detected by the system.

Standardization

The ACS:180 TSH assay is standardized against the World Health Organization (WHO) 2nd IRP 80/558 reference material.

Specimen Collection and Handling

Serum is the recommended sample type for this assay.

The following recommendations for handling and storing blood samples are furnished by the National Committee for Clinical Laboratory Standards (NCCLS):[6]

- Collect all blood samples observing universal precautions for venipuncture.
- Allow samples to clot adequately before centrifugation.
- Keep tubes stoppered and upright at all times.
- Do not use samples that have been stored at room temperature for longer than 8 hours.
- Tightly cap and refrigerate specimens at 2 to 8°C if the assay is not completed within 8 hours.
- Freeze samples at or below -20°C if the sample is not assayed within 48 hours.
- Freeze samples only once and mix thoroughly after thawing.

Before placing samples on the system ensure that:

- Samples are free of fibrin or other particulate matter.
- Samples are free of bubbles.

Reagents

For In Vitro Diagnostic Use.

CAUTION:

- Discard opened assay reagents that are at room temperature (20 to 30°C) for a total of 40 hours. Do not use these reagents to calibrate the system or assay samples.
- Do not use kit components beyond the expiration date.
- Do not mix reagents from different lots.

Reagent	Volume	Ingredients	Storage	Stability
ACS:180 TSH Lite Reagent	2.5 mL/vial	monoclonal mouse anti-TSH antibody (~833 ng/vial) labeled with acridinium ester in phosphate buffered saline with sodium azide (<0.1%) and preservatives	2–8°C	until the expiration date on the vial label or cumulative 40 hours at room temperature
ACS:180 TSH Solid Phase	11.25 mL/vial	polyclonal sheep anti-TSH antibody (~480 µg/vial) covalently coupled to paramagnetic particles in phosphate buffered saline with protein stabilizers, sodium azide (0.11%), and preservatives	2–8°C	until the expiration date on the vial label or cumulative 40 hours at room temperature

WARNING: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

BIOHAZARD: Human and/or other biological source material. Handle as if potentially infectious.

This product may contain one or more of the following materials:

- human serum or plasma, or other human source material
- biological source material of non-human origin

While each human serum or plasma donor unit used in the manufacture of this product was tested and found non-reactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2 by FDA-approved methods, all products manufactured using human or non-human source material should be handled as potentially infectious. There are no approved tests for other human and/or non-human source material. Handle this product according to established good laboratory practices.[7-9]

Preparing the Reagents

For best results, thoroughly mix the Solid Phase by inverting the vial before each use. Visually inspect the bottom of the vial to ensure that all particles are dispersed and suspended. If foaming occurs, rim the top of the vial with applicator sticks to remove the foam.

- Room temperature (20 to 30°C) equilibration is not required before use. Assay precision may be enhanced by allowing the Solid Phase and Lite Reagent to equilibrate for two hours at room temperature before use.
- Place septum caps on the Lite Reagent and Solid Phase vials.
- Do not mix reagents from different lots.

Calibrating the Assay

For detailed information about entering calibration values, refer to the system operating instructions or to the online help system.

Master Curve Calibration

The ACS:180 TSH assay requires a Master Curve calibration when using a new lot number of Lite Reagent and Solid Phase. For each new lot number of Lite Reagent and Solid Phase, use the barcode wand or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values.

Two-point Calibration Interval

The ACS:180 TSH assay requires a two-point calibration:

- every 28 days
- when changing lot numbers of assay reagents
- when replacing system components
- when quality control results are repeatedly out of range

Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, quality control samples should be assayed on each day that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

For quality control of the ACS:180 TSH assay, use Chiron Diagnostics Ligand quality control material, levels 1, 2, and 3. Refer to the product inserts for the suggested Expected Values. If the quality control results do not fall within the suggested Expected Values or within the laboratory's established values, then do the following:

- review these instructions to ensure that the assay was performed according to the procedures recommended by Chiron Diagnostics
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary, rerun the quality control samples or contact Chiron Diagnostics for more assistance

Sample Volume

This assay requires 200 µL of sample for a single determination. This volume does not include the unusable volume in the sample cup or the additional volume required when performing duplicates or other tests on the same sample.

Assay Procedure

For detailed information about operating the systems, refer to the system operating instructions or to the online help system.

Refer to *Procedural Notes* for information about analyzing samples using the optional No-Wash Protocol.

NOTE:

- If automatic tray and cup assignment is on, use the printed worklist as a guide for loading calibrators, quality control material, and patient samples into the correct tray and cup positions.
- If automatic tray and cup assignment is off and all quality control and patient samples are barcoded, load samples in any position.

1. Schedule the requested tests or profiles for each sample.
2. Prepare and load Calibrator B, if required:
   a. Prepare the low and high calibrators according to the instructions in the Calibrator B product insert.
   b. Dispense the low and high calibrators into sample cups labeled with the appropriate barcode labels.
   c. Load the sample cups on the sample tray.
   Ensure that the low calibrator precedes the high calibrator on the sample tray.
3. Prepare and load the quality control samples:
   a. Prepare the quality control material according to the instructions in the quality control product insert.
   b. Dispense the quality control samples into labeled sample cups.
   c. Load the sample cups in the appropriate positions on the sample tray.
4. Prepare the primary tubes or sample cups and load them on the sample tray.
5. If dilution is required, dispense Multi-Diluent 1 into a sample cup labeled with the appropriate barcode label and load the sample cup on the sample tray.
6. Fill a sample cup with Wash Reagent 1, label it with the appropriate barcode label, and then load the sample cup on the sample tray.
   Each test requires 200 µL of Wash Reagent 1.
7. Load the Lite Reagent and Solid Phase in adjacent positions on the reagent tray.
8. Start the system.

Procedural Notes

Calculations

For detailed information about how the system calculates results, refer to the system operating instructions or to the online help system.

The system reports serum TSH results in µIU/mL.

Dilutions

- Serum samples with TSH levels greater than 150 µIU/mL must be diluted and retested to obtain accurate results. Use Multi-Diluent 1 to dilute samples.
- Patient samples can be automatically diluted by the system or prepared manually. For automatic dilutions, ensure that Multi-Diluent 1 is loaded and set the system parameters as follows:
  Dilution setpoint: ≤ 150 µIU/mL
  Dilution factor: 2, 5
  For detailed information about automatic dilutions, refer to the system operating instructions or to the online help system.
- Manually dilute the patient samples when patient results exceed the linearity of the assay using automatic dilution, or when laboratory protocol requires manual dilution.
- Use Multi-Diluent 1 to manually dilute patient samples, and then load the diluted sample on the sample tray, replacing the undiluted sample.
- Ensure that results are mathematically corrected for dilution. If a predilution factor is entered when scheduling the test, the system automatically calculates the result.

High Dose Hook Effect

Patient samples with high TSH levels can cause a paradoxical decrease in the RLUs (high dose hook effect). In this assay, patient samples with TSH levels as high as 2000 µIU/mL will assay greater than 150 µIU/mL.

Optional No-Wash Protocol

Samples may be run in an optional No-Wash Protocol. The system uses the wash protocol according to the selected option as described in the system operating instructions or in the online help system.

When using this option, the following samples should be repeated in batch mode:

- any sample that is processed directly after a sample with a measured TSH value of 150 µIU/mL or greater
- any sample with a measured TSH value in the borderline area for hyperthyroid/euthyroid (0.3 to 0.5 µIU/mL)

Disposal

Dispose of hazardous and biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all federal, state, and local requirements.

Limitations

Interpret TSH levels with caution during the first trimester of pregnancy, or whenever very high levels of hCG are present.

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.[10] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

Serum specimens that are . . .	Have an insignificant effect on the assay up to . . .
hemolyzed	500 mg/dL of hemoglobin
lipemic	1000 mg/dL of triglycerides
icteric	40 mg/dL of bilirubin

Expected Results

To confirm the ACS:180 TSH serum reference ranges, data was obtained on 261 patients in the following categories: euthyroid (174), hypothyroid (39), and hyperthyroid (48), as classified by the alternate assay. Based on a cumulative distribution analysis of this data, the following reference ranges were established:

Clinical Condition	TSH Range (µIU/mL)
Euthyroid	0.35–5.50
Hyperthyroid	less than 0.35
Hypothyroid	greater than 5.50

As with all diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.[11]

Performance Characteristics

Specificity

The cross-reactivity of the ACS:180 TSH assay with LH, FSH, and hCG was determined by adding these hormones to human sera containing TSH. The level of TSH in the human sera was then determined.

Cross-reactant	TSH Value without cross-reactant (µIU/mL)	TSH Value with cross-reactant (µIU/mL)
LH; 200 mIU/mL	0.36	0.35
	5.39	5.12
	20.82	19.79
FSH; 200 mIU/mL	0.35	0.33
	5.12	4.99
	20.44	21.11
hCG; 200,000 mIU/mL	0.40	0.41
	5.43	5.23
	21.86	20.67

Sensitivity

The ACS:180 TSH assay measures TSH concentrations up to 150 µIU/mL with a minimum detectable concentration of 0.011 µIU/mL. Analytical sensitivity is defined as the concentration of TSH that corresponds to the RLUs that are two standard deviations greater than the mean RLUs of 27 replicate determinations of the TSH Low Calibrator (0 µIU/mL).

Assay Reportable Range

The ACS:180 TSH assay measures TSH concentrations up to 150 µIU/mL with a minimum detectable concentration of 0.011 µIU/mL.

Method Comparison

For 82 samples in the range of 0.03 to 35.33 µIU/mL, the relationship between the ACS:180 TSH assay and the Magic® Lite TSH assay is described by the equation:

ACS:180 TSH = 1.09 (Magic Lite TSH) + 0.41 µIU/mL

Correlation coefficient (r) = 0.99

Dilution Recovery

Seven human serum samples in the range of 32 to 150 µIU/mL of TSH were diluted 1:2, 1:4, 1:8, and 1:16 with Multi-Diluent 1 and assayed for recovery and parallelism. The recoveries ranged from 91% to 123% with a mean of 107%.

Precision

Three serum-based controls were assayed multiple times using one lot of reagents in 3 assays, on each of 13 systems (n = 273 for each sample), over a period of 2 days. The following results were obtained:

Mean TSH (µIU/mL)	Within-run % CV	Total % CV
0.30	4.71	6.25
4.70	3.98	5.26
15.85	3.64	4.93

Technical Assistance

For technical assistance, customers in the United States may call Chiron Diagnostics at 800-255-2121 or fax questions to the Technical Assistance Center at 508-660-4559. Customers outside the United States may contact an authorized Chiron Diagnostics representative.

For customer service or additional information, customers in the United States may call 800-255-3232. Customers outside the United States may contact an authorized Chiron Diagnostics distributor.

References

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