



Thyroid Stimulating Hormone (TSH) ELISA

For the quantitative determination of TSH in human serum.

For Research Use Only. Not For Use in Diagnostic Procedures.

Catalog Number: 25-TSHHU-E01

Size: 96 wells

Version: 11.0 2023/04-06 - ALPCO 4.1

Intended Use

For the quantitative determination of thyroid stimulating hormone (TSH) in human serum. For research use only. Note for use in diagnostic procedures.

Principle of the Assay

The TSH ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-TSH antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60-minute or overnight incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution (1 N HCl), changing the color to yellow. The concentration of TSH is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Reagents

Materials provided with the kit:

1. *Antibody-Coated Wells (1 plate, 96 wells):* Microtiter wells coated with mouse monoclonal anti-TSH antibody.
2. *Enzyme Conjugate Reagent (1 bottle, 13 mL):* Contains goat anti-TSH conjugated to horseradish peroxidase.
3. *Reference Standard Set [Standard 1-6] (1 vial per level; total 6 vials):* Contains 0, 0.5, 2, 5, 10 and 25 $\mu\text{IU/mL}$ (WHO, 2nd IRP, 80/558) TSH in equine serum with preservatives, lyophilized.
4. *TMB Reagent (1 bottle, 11 mL):* Contains 3,3',5,5' tetramethylbenzidine (TMB) stabilized in buffer solution.
5. *Stop Solution (1N HCl), 1 bottle, 11 mL* Contains diluted hydrochloric acid.

Materials required but not provided with the kit:

1. Distilled or deionized water
2. Precision pipettes: 50 μL , 100 μL , 200 μL , and 1 mL
3. Disposable pipette tips
4. Vortex mixer or equivalent
5. Microtiter well reader capable of reading absorbance at 450nm
6. Absorbent paper
7. Graph paper (semi-log, etc.)
8. Quality control material (e.g., BioRad Lyphocheck Control sera)
9. Timer

Warnings and Precautions

1. **Caution:** This kit contains human material. The source material used to manufacture this kit tested negative for HBsAg, HIV 1/2, and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate local and/or national biohazard safety guideline or regulation.

2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use reagents when they become cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with plenty of water and seek medical attention if irritation persists.
10. For research use only. Not for use in diagnostic procedures.

Storage of the Kit

Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date. Opened and used reagents, with the exception of the lyophilized standards, are stable until the expiration date if stored properly at 2-8°C. Reconstituted standards are stable for up to 30 days when stored sealed at 2-8°C. Keep the microtiter plate in a sealed bag with desiccants to minimize exposure to damp air.

Instrumentation

A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

Sample Collection and Preparation

Serum should be prepared from a whole blood sample obtained by acceptable techniques. This kit is for use with serum samples without additives only. Avoid grossly hemolytic, lipemic, or turbid samples. Samples should be capped and may be stored for up to 48 hours at 2-8°C prior to assay use. Samples held for a longer time should be frozen only once at -20°C prior to use. Thawed samples should be inverted several times prior to testing.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Reconstitute each lyophilized standard with 1.0mL deionized water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.
4. Samples expected to have a TSH concentration greater than 25 µIU/mL should be diluted 1:10 with TSH-free serum (Zero calibrator).

Procedural Notes

Pipetting of all standards, samples, and controls should be completed within 3 minutes. Use of a multichannel pipette is recommended.

All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.

It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μ L of standards, samples, and controls (not included in kit, see Materials Required but Not Provided Section) into appropriate wells.
3. Dispense 100 μ L of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature (18-25°C) for 60 minutes (1 hour).
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or lint-free paper towels to remove all residual water droplets.
9. Dispense 100 μ L of TMB reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 μ L of Stop Solution to each well.
12. Gently mix for 30 seconds. **Ensure that all of the blue color changes completely to yellow.**
13. Read absorbance at 450nm with a microtiter plate reader within 15 minutes.

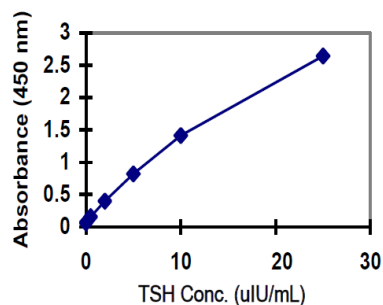
Calculations of Results

1. Calculate the mean absorbance value (OD450) for each set of reference standards, controls, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in μ IU/mL, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of TSH in μ IU/mL from the standard curve.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

Example of Standard Curve

Results of a typical standard run of the assay are shown below. The standard curve is for illustration only, and should not be used to calculate unknowns. The standard curve covers a dynamic range from 0 to 25 μ IU/mL. The absorbance (450 nm) value can vary due to incubation at different room temperatures in different laboratories.

TSH (μ IU/mL)	Absorbance (450nm)
0	0.063
0.5	0.157
2	0.398
5	0.818
10	1.415
25	2.645



PERFORMANCE CHARACTERISTICS

Sensitivity

At TSH concentrations of 0.1 $\mu\text{IU/mL}$ and 0.2 $\mu\text{IU/mL}$, the inter-assay CVs were determined to be 11.4% and 7.9%, respectively.

Precision

Intra-Assay Precision

Within-run precision was determined by replicate determinations of four different serum samples in one assay. Within-assay variability is shown below:

Serum Sample	1	2	3	4
Number of Replicates	28	26	26	26
Mean TSH ($\mu\text{IU/mL}$)	0.62	1.51	15.48	26.14
Standard Deviation	0.03	0.09	0.39	0.86
CV(%)	4.6%	5.7%	2.5%	3.3%

Inter-Assay Precision

Between-run precision was determined by replicate determinations of four different serum samples over a series of individually-calibrated assays as shown below:

Serum Sample	1	2	3	4
Number of Replicates	30	24	24	24
Mean TSH ($\mu\text{IU/mL}$)	0.64	1.46	15.38	25.26
Standard Deviation	0.05	0.10	0.87	1.75
CV(%)	7.6%	7.1%	5.7%	8.9%

Recovery and Linearity

Recovery

Various serum samples of known TSH levels were combined and assayed in duplicate. The mean recovery was 98.9%.

Expected Concentration ($\mu\text{IU/mL}$)	Observed Concentration ($\mu\text{IU/mL}$)	% Recovery
23.94	22.92	95.7%
11.75	11.01	93.6%
5.46	5.53	101.3%
2.76	3.04	110.1%
1.48	1.39	93.9%

Linearity

Two samples were serially diluted with zero standard to determine linearity. The mean recovery was 94.6%.

Sample	Dilution	Expected Conc. (μ IU/mL)	Observed Conc. (μ IU/mL)	% Expected
1	Undiluted	-	48.39	-
	1:2	24.2	22.43	91.9%
	1:4	11.21	10.45	93.2%
	1:8	5.6	5.13	91.6%
	1:16	2.8	2.87	102.5%
	1:32	1.4	1.3	92.9%
			Average = 94.4%	
2	Undiluted	-	36.42	-
	1:2	18.21	18.1	99.4%
	1:4	9.1	8.48	93.2%
	1:8	4.55	4.28	94.1%
	1:16	2.27	2.22	97.8%
	1:32	1.13	1.01	89.4%
			Average = 94.8%	

Specificity

The following hormones were tested for cross-reactivity:

Hormone	Concentration	Produced Intensity Equivalent to TSH (μ IU/mL)
HCG – (WHO 2 nd IS 61/6)	100 mIU/mL	0
	600 mIU/mL	0
	3,500 mIU/mL	0
	10,000 mIU/mL	0
	200,000 mIU/mL	0
FSH – (WHO 2 nd IRP-HMG)	20 mIU/mL	0
	100 mIU/mL	0
	200 mIU/mL	<0.2
LH – (WHO 1 st IRP 68/40)	75 mIU/mL	0
	150 mIU/mL	0
	300 mIU/mL	<0.2
Prolactin – (WHO – 1 st IRP 75/504)	10 ng/mL	0
	50 ng/mL	0
	200 ng/mL	<0.2
hGH – (WHO 1 st IRP 65/217)	10 ng/mL	0
	50 ng/mL	0
	200 ng/mL	<0.2

Hook Effect

No hook effect is observed at TSH concentrations up to 1,000 μ IU/mL.

QUALITY CONTROL

Good laboratory practice requires that low, medium, and high quality control samples (controls) be run with each calibration curve to verify assay performance. To ensure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

STANDARDIZATION

The TSH reference standards are calibrated against the WHO 2nd International Reference Preparation of hTSH 2nd IRP-80/558.

LIMITATIONS

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. For professional use only.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this assay.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.