DRG® Ultrasensitive TSH (U-TSH) ELISA (EIA-1790)

CE

Revised 28 Dec. 2009 rm (Vers. 4.1) **IVD**

Please use only the valid version of the package insert provided with the kit.

For In Vitro Diagnostic Use

Store at 2 to 8°C.

I. Intended Use

For the quantitative determination of thyroid stimulating hormone (TSH) concentration in human serum. The assay is useful in the diagnosis of thyroid or pituitary disorders.

II. Introduction

The determination of serum or plasma levels of thyroid stimulating hormone (TSH or thyrotropin) is recognized as an important measurement in the assessment of thyroid function.[1] Thyroid stimulating hormone is secreted by the anterior lobe of the pituitary gland, and induces the production and release of thyroxine (T4) and triiodothyronine (T3) from the thyroid gland.[2] It is a glycoprotein with a molecular weight of approximately 28,000 daltons, consisting of two chemically different subunits, alpha and beta.[3]

Although the concentration of TSH in the blood is extremely low, it is essential in the maintenance of normal thyroid function. The release of TSH is regulated by a TSH-releasing hormone (TRH) produced by the hypothalamus. The levels of TSH and TRH are inversely related to the level of thyroid hormone. When there is a high level of thyroid hormone in the blood, less TRH is released by the hypothalamus, so less TSH is secreted by the pituitary. The opposite action will occur when there are decreased levels of thyroid hormones in the blood. This process, known as a negative feedback mechanism, is responsible for maintaining the proper blood levels of these hormones.[4,5]

Conventional TSH assays are generally accepted as an important tool in the diagnosis of primary and secondary hypothyroidism1, but offer limited clinical utility in the assessment of hyperthyroidism (overactive thyroid) due to a lack of sensitivity. The Ultrasensitive-TSH ELISA (U-TSH) offers a sensitivity of 0.05 µIU/mL and hence allows discrimination between hyperthyroid and normal patient populations. The assay is intended to quantitatively measure TSH in human serum with 2nd generation sensitivity. The U-TSH ELISA can be used as an aid in the assessment of thyroid status, and diagnosis and treatment of thyroid disease.

III. Principle of the Assay

The Ultrasensitive-TSH ELISA Test is based on the principle of a solid phase enzyme-linked immunosorbent assay.[6,7] 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 fore solid phase (microtiter wells) immobilization, and goat anti-TSH antibody is used in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the TSH molecule being sandwiched between the solid phase and enzyme-linked antibodies. After a 2 hour incubation at room temperature with shaking, the solid phase is washed with distilled water to remove unbound labeled antibodies. A solution of tetramethylbenzidine (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 1N HCl, and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of TSH is directly proportional to the color intensity of the test sample.

IV. Reagents and Materials Provided

1. Antibody-Coated Wells (1 plate, 96 wells)

Microtiter wells coated with mouse monoclonal anti-TSH.

2. Enzyme Conjugate Reagent (1 vial, 13 mL)

Contains goat anti-TSH conjugated to horseradish peroxidase.

3. Reference Standard Set (1 mL/vial)

Contains 0, 0.1, 0.5, 2.0, 5.0 and 10.0 µIU/mL (WHO, 2nd IRP, 80/558)
TSH in equine serum with preservatives.
Lyophilized. See instructions for reconstitution under Reagent Preparation.

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.

V. Materials Required But Not Provided

1. Distilled or deionized water
2. Precision pipettes: 0.05, 0.1, 0.2, and 1 ml
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450 nm.
5. Orbital motion microtiter well shaker, capable of shaking at a speed of 175 ± 25 RPM
6. Absorbent paper
7. Log-log graph paper
8. Quality control material (e.g., BioRad Lyphochek Control sera)

VI. Warnings and Precautions

1. CAUTION: Test methods are not available which can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[8]

2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date.
4. Do not mix or use components from kits with different lot numbers.
5. Replace caps on reagents immediately. Do not switch caps.
6. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
7. Do not pipette reagents by mouth.
8. For in vitro diagnostic use.

VII. Storage Conditions

1. 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.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

VIII. Instrumentation

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.
An orbital motion microtiter plate shaker is necessary for the 2 hour incubation.

IX. Specimen Collection and Preparation

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

X. Procedural Notes

1. Manual Pipetting:
   It is recommended that no more than 32 wells be used for each assay run.
   Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. Automated Pipetting:
   A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.

3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

XI. 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 1.0 mL dH_20.
   Allow the reconstituted material to stand for at least 20 minutes.
   Reconstituted standards should be stored sealed at 2-8°C, and are stable at 2-8°C for at least 30 days.

XII. Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µL of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing.
5. Incubate at room temperature (18-25°C) with shaking at 175 ± 25 RPM for 120 minutes (2 hours).
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled or dionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µL of TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature, for 20 minutes.
11. Stop the reaction by adding 100 µL of Stop Solution into each well.
12. Gently mix for 30 seconds. ***Ensure that all of the blue color changes completely to yellow.***
13. Read OD at 450 nm with a microtiter well reader **within 15 minutes.**

XIII. Calculation of Results

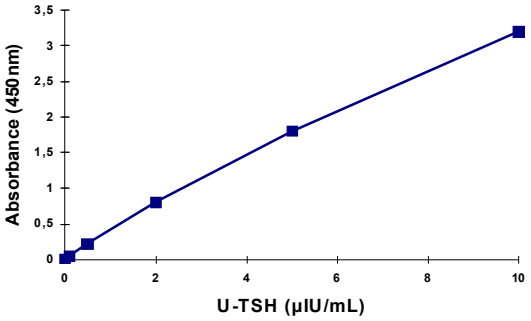
1. Calculate the average absorbance value (A450) for each set of reference standards, controls and samples.
2. Using log-log graph paper, construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in µIU/mL, with absorbance on the vertical (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 µIU/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

4. Any diluted samples must be further corrected by the appropriate dilution factor.

A. Example of Standard Curve

Results of a typical standard run with absorbency readings at 450nm shown in the Y axis against TSH concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns.

TSH (µIU/mL)	Absorbance (450 nm)
0	0.009
0.1	0.050
0.5	0.218
2	0.801
5	1.800
10	3.191



XIV. Expected Values

Each laboratory should establish its own normal range based on patient population. Differences in assay technique and the use of various standards may affect results.

The results provided below are based on 43 normal and 73 hyperthyroid blood specimens. The ranges were determined from the mean ± 2SD (µIU/mL TSH). These values may differ from other published data.

	Normal	Hyperthyroid
N	43	73
Mean TSH (µIU/mL)	1.84	< 0.07
Range	0.54 - 4.72	< 0.07 – 0.20

XV. Performance Characteristics

A. Accuracy

The U-TSH ELISA (EIA-1790) has been compared to the Diagnostic Products Corporation's Immulite 2000 3rd Generation TSH test and is substantially equivalent in that the methods used are for the quantitative determination of TSH in human serum. Likewise, both methods use TSH calibrated and labeled in µIU/ml standardized to the WHO hTSH (2nd IRP, 80/558).

A study was conducted using 62 euthyroid and hypothyroid patient samples (as determined by hospital laboratory analysis). The range of samples tested was 0.4 µIU/mL to 62 µIU/mL. The comparison demonstrated good correlation with another commercial kit as shown below:

N	= 62
Correlation coefficient	= 0.997
Slope	= 1.032
Intercept	= 0.240
EIA-1790 Mean	= 8.31 µIU/mL
DPC Mean	= 8.82 µIU/ml

Another 73 hyperthyroid patient samples also correlated well with the DPC test kit:

	# Samples [TSH] ≤ 0.1 µIU/mL	**# Samples [TSH] ≤ 0.4 µIU/mL [TSH] > 0.1 µIU/mL**
DPC Kit	N = 71	N = 2
EIA-1790 Kit	N = 67	N = 6

Note: The six patient samples with TSH concentration greater than 0.1 µIU/mL in the EIA-1790 test had concentrations of less than 0.4 µIU/ml, which is still in the hyperthyroid range (< 0.4 µIU/mL).

B. Sensitivity

Based on the 20% interassay CV of two low-end TSH data-points (0.07 µIU/mL and 0.1 µIU/mL), the functional sensitivity of the U-TSH assay was determined to be 0.054 µIU/mL.[9]

C. Precision

1. Intra-Assay Precision

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

Serum Sample	1	2	3	4	5
Number of Replicates	20	20	20	20	20
Mean TSH (µIU/mL)	0.11	0.26	0.49	3.90	9.68
Standard Deviation	0.01	0.02	0.04	0.25	0.74
Coefficient of Variation (%)	9.2	7.7	8.2	6.4	7.6

2. Inter-Assay Precision

Between-run precision was determined by replicate measurements of five different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Serum Sample	1	2	3	4	5
Number of Replicates	30	30	30	30	30
Mean TSH (µIU/mL)	0.11	0.31	0.48	3.96	8.89
Standard Deviation	0.02	0.04	0.04	0.31	0.68
Coefficient of Variation (%)	12.9	12.9	9.8	7.8	7.6

D. Recovery and Linearity Studies

1. Recovery

For each concentration, two patient serum samples of known TSH levels were combined and assayed in duplicate. The average recovery was 104%.

Expected Concentration (µIU/mL)	Observed Concentration (µIU/mL)	% Recovery
1.14	1.21	106%
1.65	1.75	106%
3.03	2.98	98%
4.56	4.58	100%
6.05	6.40	106%
13.20	13.90	105%

2. Linearity

Four samples were serially diluted with sample diluent to determine linearity. The average recovery was 97.3%.

#	Dilution	Expected Conc. µIU/mL)	Observed Conc. µIU/mL)	% Expected
1.	Undiluted		14.6	
	1:2	7.30	7.26	99%
	1:4	3.65	3.38	93%
	1:8	1.83	1.66	91%
	1:16	0.91	0.79	87%
	1:32	0.46	0.39	85%
	1:64	0.23	0.19	83%
			Average = 90%	
2.	Undiluted		14.2	
	1:2	7.10	7.22	102%
	1:4	3.55	3.41	96%
	1:8	1.78	1.95	110%
	1:16	0.89	1.00	112%
	1:32	0.44	0.46	105%
	1:64	0.22	0.23	105%
	1:128	0.44	0.09	82%
			Average = 102%	
3.	Undiluted		3.49	
	1:2	1.75	1.80	103%
	1:4	0.87	0.93	107%
	1:8	0.44	0.48	109%
	1:16	0.22	0.24	109%
	1:32	0.11	0.11	100%
			Average = 106%	
4.	Undiluted		2.56	
	1:2	1.28	1.19	93%
	1:4	0.64	0.62	97%
	1:8	0.32	0.29	91%
	1:16	0.16	0.14	88%
	1:32	0.08	0.07	88%
			Average = 91%	

E. Specificity

The following hormones were tested for cross-reactivity:

HORMONE TESTED	CONCENTRATION	PRODUCED INTENSITY EQUIVALENT to TSH (µIUmL)
HCG - (WHO 1st IRP 75/537)	1,000 mIU/mL	0
	5,000 mIU/mL	0
	10,000 mIU/mL	0
	50,000 mIU/mL	0
	100,000 mIU/mL	0
	250,000 mIU/mL	0
	500,000 mIU/mL	0
FSH - (WHO 2nd IRP-HMG)	100 mIU/mL	0.00
	250 mIU/mL	0.00
	500 mIU/mL	0.10
	750 mIU/mL	0.18
LH - (WHO 1st IRP 68/40)	100 mIU/mL	0.06
	250 mIU/mL	0.13
	500 mIU/mL	0.15
	750 mIU/mL	0.18
Prolactin - (WHO 1st IRP 75/504)	100 ng/mL	0
	250 ng/mL	0
	500 ng/mL	0
	750 ng/mL	0
	1000 ng/mL	0
hGH - (WHO 1st IRP 65/217)	50 ng/mL	0
	100 ng/mL	0
	200 ng/mL	0
	400 ng/mL	0

F. Hook Effect

No hook effect is observed in this assay at TSH concentrations up to 1,000 µIU/mL.

XVI. Quality Control

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To assure 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.

XVII. Standardization

The TSH Reference Standards are calibrated against the World Health Organization's Second International Reference Preparation of hTSH 2nd IRP-80/558).

XVIII. Limitations of the Procedure

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. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. The EIA-1790 U-TSH assay has not been tested on newborns, and is not for use in screening newborns.

XIX. References

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