DRG

DRG® TSH (CLA-4658)

CE

USA: RUO

REVISED 4 JAN. 2007

1 INTENDED USE

For the direct quantitative determination of Thyroid Stimulating Hormone in human serum by chemiluminescence immunoassay (CLIA). **For *in vitro* use only.**

2 PRINCIPLE OF THE TEST

The principle of this chemiluminescence immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for TSH is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of TSH is conjugated to horse radish peroxidase (HRP). TSH from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. After the washing step, the luminescence substrate is added and the relative luminescence units (RLUs) are measured in a microtitre plate luminometer. The RLUs formed by the enzymatic reaction are directly proportional to the concentration of TSH in the samples. A set of standards is used to plot a standard curve from which the concentration of TSH in patient samples and controls are read.

3 CLINICAL APPLICATIONS

Thyroid stimulating hormone (TSH) is a glycoprotein hormone of 28KD secreted by the anterior pituitary gland. TSH has two subunits, namely α and β. The α subunit of TSH is similar to the α subunit found in the LH, FSH and hCG glycoprotein hormones. However, the β subunit is specific and differs from hormone to hormone.

TSH stimulates positively the production of thyroid hormones T4 and T3. Circulating T4 and T3 regulate the TSH secretion by negative feedback. TSH production is also under the positive control of thyrotropin-releasing hormone (TRH), which is secreted by hypothalamus.

Measurement of serum TSH is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary hypothyroidism. In primary hypothyroidism, where there is impaired production of thyroid hormones, the TSH level is typically highly elevated. In secondary or tertiary hypothyroidism where the thyroid hormones are low as a consequence of pituitary or hypothalamic lesions, the TSH level is usually low.

Further, a sensitive TSH assay is also able to differentiate the hyperthyroidism from the euthyroid population. TSH is typically suppressed to subnormal levels in most hyperthyroidism

It is recommended to assay both TSH and thyroid hormones for the clinical assessment of thyroid status. But if there were to be only one test to be prescribed for thyroid function, TSH would be the test. TSH determinations are also helpful to monitor patients who receive thyroxine replacement therapy.

4 PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.

2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The kit control should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
9. The luminescence substrate solutions (A and B) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
10. When dispensing the substrate, do not use pipettes in which this liquids will come into contact with any metal parts.
11. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
12. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
13. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

5 LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of TSH in human serum. The kit is not calibrated for the determination of TSH in saliva, plasma or other specimens of human or animal origin. The kit is not indicated for use with neonatal blood spot for newborn screening,
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.
6. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

6 SAFETY CAUTIONS AND WARNINGS

Human serum that may be used in the preparation of the standards and control has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid direct contact with reagents. In case of contact, wash with plenty of water.

7 SPECIMEN COLLECTION AND STORAGE

Approximately 0.2 ml of serum is required per duplicate determination. Collect 4-5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer.

Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

8 SPECIMEN PRETREATMENT

This assay is a direct system; no pretreatment of serum is necessary.

9 REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 50, 100 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. A plate shaker
5. Plate shaker
6. Microwell plate luminometer

10 REAGENTS PROVIDED AND PREPARATION

1. Mouse Anti-TSH Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use.

Contents: One 96 well (12x8) monoclonal antibody-coated microwell plate in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

2. Mouse Anti-TSH Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate - Requires Preparation.

Contents: Anti-TSH monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.

DRG

DRG® TSH (CLA-4658)

CE

USA: RUO

REVISED 4 JAN. 2007

Volume: 300 µL/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µL of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µL of HRP in 12ml of assay buffer. Discard any that is left over.

3. TSH Calibrators - Ready To Use.

Contents: Seven vials containing TSH in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of TSH. Calibrated against World Health Organization (WHO) 2nd IS 80/558.

*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator A	0 µIU/ml	2.0 ml
Calibrator B	0.008 µIU/ml	0.5 ml
Calibrator C	0.04 µIU/ml	0.5 ml
Calibrator D	0.2 µIU/ml	0.5 ml
Calibrator E	1.0 µIU/ml	0.5 ml
Calibrator F	5.0 µIU/ml	0.5 ml
Calibrator G	25.0 µIU/ml	0.5 ml

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Control - Ready To Use.

Contents: One vial containing TSH in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of TSH. Refer to vial label for expected value and acceptable range.

Volume: 0.5 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vial or as indicated on label. Once opened, the control should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate - Requires Preparation.

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 ml/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

6. Assay Buffer - Ready To Use.

Contents: One vial containing a protein-based buffer with a non-mercury preservative.

Volume: 15 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

7. Cheminluminescence Substrate Reagent A - Requires Preparation.

Contents: One bottle containing luminol enhancer.

Volume: 1 ml/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: See below.

8. Cheminluminescence Substrate Reagent B - Requires Preparation.

Contents: One vial containing peroxide solution.

Volume: 1 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: See below.

9. Cheminluminescence Substrate Reagent C - Requires Preparation.

Contents: One vial containing buffer with a non-mercury preservative.

Volume: 15 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: See below.

Preparation of Working Substrate Solution:

Mix **1 part** of the chemiluminescence **substrate reagent A** with **1 part of reagent B** and **10 part of reagent C**. This gives the ready to use substrate solution. Prepare fresh for each use.

If the whole plate is to be used prepare working substrate solution as follows:

Combine 1 ml of reagent A with 1 ml of reagent B and 10 ml of reagent C.
Total volume=12 ml of working substrate solution.

Stability: Working substrate solution is stable for 24 hours at room temperature.

11 ASSAY PROCEDURE

Specimen Pretreatment: *None.*

Important Notes:

1. All reagents must reach room temperature before use.
2. Once the procedure has been started, all steps should be completed without interruption to ensure equal elapsed time for each pipetting step.
3. The washing procedure influences the precision markedly; it is essential to ensure the washing is effective and thorough.

Procedure:

1. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
2. Pipette 50 µL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
3. Pipette 100 µL of the conjugate working solution into each well
   (We recommend using a multichannel pipette).
4. Cover the plate and incubate for 30 minutes on a plate shaker at room temperature.
5. Wash the wells 5 times, each time with 300 µL of diluted wash buffer per well and on the last wash tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
6. Pipette 100 µL of chemiluminescence working substrate solution into each well
   (We recommend using a multichannel pipette).
7. Incubate on a plate shaker (approximately 200 rpm) for 10-15 minutes at room temperature.
8. Measure the RLUs in each well on a plate luminometer within 20 minutes after addition of the substrate.

12 CALCULATIONS

1. Calculate the mean RLU of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean RLUs on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean RLU of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 25 µIU/ml then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

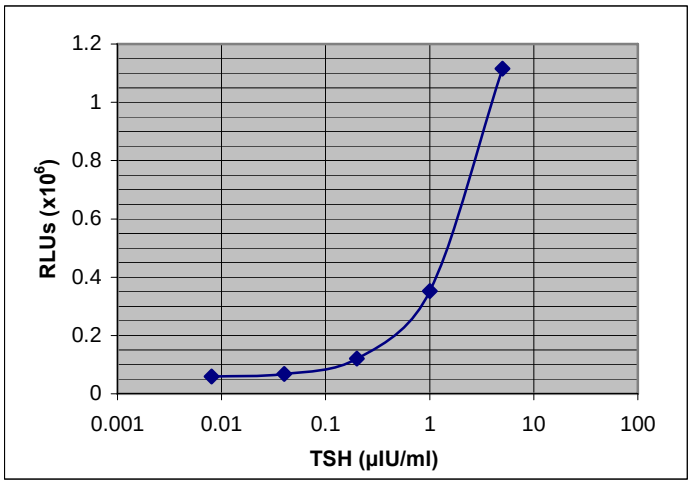
*TYPICAL TABULATED DATA***

Calibrator	RLU 1 x 10^3	RLU 2 x 10^3	Mean RLU x 10^3	RLU/RLU_{MAX} (%)
A, 0 µIU/ml	50	52	51	2.5
B, 0.008 µIU/ml	57	61	59	2.8
C, 0.04 µIU/ml	66	69	68	3.3
D, 0.2 µIU/ml	121	120	121	6
E, 1 µIU/ml	368	336	352	17
F, 5 µIU/ml	1144	1158	1151	56
G, 25 µIU/ml	1948	2142	2045	100

**- It is recommended to use the RLU/RLU_{MAX} values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show quite different RLU values, however, the RLU/RLU_{MAX} values remain consistent.

TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



13 PERFORMANCE CHARACTERISTICS

13.1 SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean RLU of Calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the DRG® Direct TSH CLIA kit is **0.002µIU/ml.**

13.2 SPECIFICITY (CROSS REACTIVITY)

The specificity of the TSH ELISA kit was determined by measuring the apparent TSH values of the following compounds:

Substance	Concentration Range	Apparent TSH Value (µIU/ml)
HCG Calibrated against WHO 1st IS 75/537	10,000-50,000 IU/L	<0.15
HFSH Calibrated against WHO 1st 83/575	1000-4000 IU/L	<0.15
HLH Calibrated against WHO 2nd IS 80/552	100-500 IU/L	<0.15

13.3 INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in µIU/ml) are tabulated below:

Sample	Mean	SD	CV%
1	0.16	0.009	5.631
2	1.01	0.057	5.650
3	11.52	0.989	8.590

13.4 INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in µIU/ml) are tabulated below:

Sample	Mean	SD	CV%
1	0.178	0.019	10.68
2	1.339	0.105	7.84
3	10.10	0.978	9.68

13.5 RECOVERY

Spiked samples were prepared by adding an exact amounts of TSH to four patient serum samples. The results (in µIU/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	1.07	-	-
+3.16	4.15	4.23	98.1
2 Unspiked	0.08	-	-
+0.1	0.16	0.18	88.9
+1.0	1.01	1.08	93.5
+10.0	11.52	10.08	114.3
3 Unspiked	1.58	-	-
+3.07	3.71	4.65	80.0
+5.26	5.62	6.84	82.2
+6.51	6.49	8.09	80.2
4 Unspiked	1.24	-	-
+3.07	3.68	4.31	85.4
+5.26	5.79	6.50	89.1
+6.51	6.62	7.75	85.4

13.6 LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in µIU/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	4.15	-	-
1:2	1.86	2.08	89.4
1:4	1.08	1.04	103.8
1:8	0.56	0.52	107.7
2	3.08	-	-
1:2	1.56	1.54	101.3
1:4	0.85	0.77	110.4
1:8	0.47	0.39	120.5
3	11.06	-	-
1:2	5.44	5.53	97.6
1:4	2.98	2.76	108.0
1:8	1.74	1.38	126.1

13.7 COMPARATIVE STUDIES

The DRG®'s TSH CLIA assay was compared to a TSH ELISA method on 20 samples (concentration range: 0.64 – 5.56 µIU/ml).

By linear regression:

(This method) = 0.92 x (ELISA) + 0.14 uIU/ml; r = 0.986

Means: CLIA: 1.87 uIu/ml

ELISA: 2.03 UIU/ml

14 EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (µIU/ml)
Normal	0.3 - 5
Hyperthyroid	< 0.15
Hypothyroid	> 5.7

15 REFERENCES

1. Allen, K. R., et al., Ann.Clin.Biochem. 22:506, 1985
2. Benkirane, M., et al., J.Immunol.Meth. 98:173, 1987
3. Carayon, P., et al., Hormone Res. 26:105, 1987
4. Carayon, P., et al., Ann. Endocrinol. 40:211, 1979
5. Clark, P.M.S., et al., Clin. Chem. 32:88, 1986
6. Cornell, J. S., et al., J. Biol. Chem. 248:4327, 1978
7. Cusick, C. F., et al., Clin. Chem. 31:348, 1985
8. Dumont, J.E., Vitamins Horm. 29:287, 1971
9. Dumont, J.E., In Endocrinology(ed:de Groot, L.J., Grune and Stratton Vol. 1. 311-329, 1979
10. Evans, M. C., et al., Clin. Endocrinol. 22:445, 1985
11. Greenspan, F. S., et al., J. Clin. Endo. Metab. 38:1121, 1974
12. Hall, R., et al., Br. Med. J. 1:582, 1971
13. Howanitz, P. J., et al., Clin. Chem. 28:427, 1982
14. Lower, E. G., et al., Endocrine Rev. 4:213, 1983
15. Malter, J. S., et al., Clin. Chem. 31:642, 1985

DRG® TSH (CLA-4658)

USA: RUO

REVISED 4 JAN. 2007

SYMBOLS USED WITH DRG ELISAS

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	Research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..

DRG International Inc., USA

Fax: (908) 233-0758 • E-mail: corp@drg-international.com • **Web:** www.drg-international.com