



TSH-R Ab

BL-16-100

**RADIORECEPTOR ASSAY FOR QUANTITATIVE DETERMINATION OF
ANTI-TSH RECEPTORS AUTOANTIBODIES IN HUMAN SERUM
100 Determinations**

FOR IN VITRO DIAGNOSTIC USE ONLY

GENERAL INFORMATION

- A. Name: **Bio-Line** TSH-R Ab.Kit
- B. Catalogue number: BL-16-100: 100 tests
- C. Manufactured by: Bio-Line S.A.

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1. CLINICAL APPLICATION

Hyperthyroidism in Graves' disease is due to auto-antibodies directed against the thyrotropin (TSH) receptor and measurement of these auto-antibodies is valuable in the diagnosis and management of the Graves' disease.

2. PRINCIPLE OF THE ASSAY

The TSH RECEPTOR AUTO-ANTIBODIES assay is a Radioreceptor Assay (RRA) which detects autoantibodies that interfere with the binding of thyroid stimulating hormone (TSH) to TSH receptor. In the assay, TSH receptor is incubated with serum, and after a pre-incubation time ^{125}I -labelled TSH (tracer). Autoantibodies and tracer compete for binding sites on the receptor. Consequently, the higher the autoantibody titer, the lower the ^{125}I TSH bound to the receptor. Following incubation the receptor-tracer complex is precipitated with polyethylene glycol (PEG). After centrifugation, the supernatant is removed and the precipitate counted. The units of TSH RECEPTOR AUTO-ANTIBODIES activity in the sample are determined from the calibration curve and expressed as units/L (U/L).

3. REAGENTS PROVIDED WITH THE KIT

- The reagents are sufficient for 50 (100) determinations.
- Store the kit and reagents at 2-8 °C.
- The expiration date of each reagent is shown on the label.

- 1. Radioactive Tracer :** 2 (4) vials containing 2.8 ml (lyophilised) of purified ^{125}I -bTSH. Reconstitute each vial with 2.8 ml of distilled water and mix gently. Reconstitute the tracer at least 10 min before the beginning of the test. Any excess solution not used in the test must be discarded. Radioactivity content: 12.8 KBq per vial. Preservative: NaN_3 (<0.1%).
- 2. Calibrators: Calibrator 0 :** 1 vial containing 1 ml human serum. Preservative: NaN_3 (<0.1%). **Calibrators 1-5:** 5 vials containing 0.5 ml bTSH in human serum. Preservative: NaN_3 (<0.1%). The calibrators contain 5 – 15 – 45 – 135 – 405 U/L. Calibrators have been standardised in the receptor assay against MRC LATS Standard B. 1 unit of calibration is approximately equivalent to 1 unit of MRC LATS standard B.
- 3. Controls:** 2 vials containing 0.5 ml of serum. Preservative: NaN_3 (<0.1%). **For the exact value, refer to the value written on the Quality Control data sheet.**
- 4. TSH receptor:** 2 (4) vials containing lyophilised TSH receptors. Preservative: NaN_3 (<0.1%). Reconstitute each vial with 1.4 ml of cold buffer, mix gently and allow to dissolve for 10 min until the content is uniformly suspended. Mix the contents on a vortex mixer set at full speed to ensure dissolution of the receptors. Do not reconstitute vials which will not be used immediately in the assay. Reconstituted TSH Receptors are not very stable. Any excess solution not used in the test must be discarded.
- 5. NSB (control receptor):** 1 vial containing 1 ml of NSB solution. Preservative: NaN_3 (<0.1%).
- 6. Buffer:** 1 vial containing 6.5 ml of buffered solution for reconstitution of TSH receptors. Preservative: NaN_3 (<0.1%). Always use a cold solution for reconstitution of TSH receptors.
- 7. PEG:** 1(2) vials of buffered solution containing 100 ml of PEG. Preservative: NaN_3 (<0.1%).

4. MATERIAL REQUIRED BUT NOT SUPPLIED.

- Conical test tubes (12 x 75 mm).
- Test tube racks.
- Graduated cylinder.
- Distilled water.
- Adjustable, automatic micropipettes with disposable tips.
- Vortex mixer.
- Orbital shaker
- Aspiration pump or automated tube washing device.
- Refrigerated centrifuge (min 2500 x g)
- Scintillation gamma counter.

5. WARNINGS AND PRECAUTIONS

In order to obtain reproducible results, the following rules must be observed:

- Do not mix reagents of different lots.
- Do not use reagents beyond their expiry date.
- Use thoroughly clean glassware.
- Use distilled water, stored in clean containers.
- Avoid any contamination among samples; for this purpose, disposable tips should be used for each sample and reagent.

In order to avoid personal and environmental contamination, the following precautions must be observed:

- Use disposable gloves while handling potentially infectious material and performing the assay.
- Do not pipette reagents by mouth.
- Do not smoke, eat, drink or apply cosmetics during the assay.
- All material of human origin used for the preparation of this kit tested negative for HBsAg, anti-HIV and anti-HCV. Since no test at present can guarantee complete absence of these viruses, all samples and reagents used for the assay must be considered potentially infectious therefore, the assay waste must be decontaminated and disposed of, in accordance with established safety procedures. Disposable ignitable material must be incinerated; disposable non-ignitable material must be sterilized in autoclave for at least 1 hour at 121 °C. Liquid wastes must be added with sodium hypochlorite at a final concentration of 3%. Let the hypochlorite act for at least 30 minutes. Liquid wastes containing acid must be neutralized with appropriate amounts of base, before treating with sodium hypochlorite.
- Avoid splashing and aerosol formation; in case of spilling, wash carefully with a 3% sodium hypochlorite solution and dispose of this cleaning liquid as potentially infectious waste. Some reagents contain sodium azide as preservative; to prevent build-up of explosive metal azides in lead and copper plumbing, reagents should be discarded by flushing the drain with large amounts of water.
- Acquisition, storage, use and disposal of radioactive material (liquid and solid) are subject to regulation and ordination of local authorities.

6. SPECIMEN COLLECTION

Sera to be analysed must be assayed soon after separation or stored in aliquotes at -20 °C. Freezing and thawing must be avoided.

Incorrect storage of samples can lead to loss of TSH RECEPTOR AUTO-ANTIBODIES activity.

Do not use grossly hemolyzed or lipemic samples.

Do not use plasma in the assay.

When required, thaw the samples at room temperature and mix gently by inversion to ensure homogeneity.

Centrifuge the samples prior assay (preferably at 1,000-1,500 g in a microfuge) to remove any particulate matter.

7. ASSAY PROCEDURE WITH OR WITHOUT CALIBRATORS

- Use of conical polystyrene tube is strongly recommended. Label the tubes for Total counts (T), Non Specific Binding (NSB), Calibrator 0 (Bo), Calibrators, Controls, and Samples in duplicate.
- NSB:** pipette 50 μl of NSB solution into the corresponding tubes + 50 μl of Calibrator 0.
- CAL 0:** pipette 50 μl of calibrator 0 into the corresponding tubes.
- CALIBRATION CURVE:** pipette 50 μl of each calibrator solution into their respective tubes.
In assay procedure without calibrators do not perform this step 4.
- SAMPLES AND CONTROLS:** pipette 50 μl of Control or patient's serum into their respective tubes.
- Add 50 μl of TSH RECEPTOR solution to all except to the "Total count" tubes and NSB tubes. Mix all tubes with a "vortex mixer".
- Incubate for 10 minutes at room temperature (on orbital shaker at 150 rpm)
- Add 100 μl of ^{125}I - bTSH tracer to each tube and mix all tubes with a "vortex mixer".
- Incubate at room temperature for 2 hours (on orbital shaker at 150 rpm).
- Agitate the PEG solution by magnetic stirring and add 2 ml of cold PEG solution (4 °C) to each tube except for "Total count" tubes.

IMPORTANT:

AGITATE EACH TUBE THOROUGHLY USING A "VORTEX MIXER"

11. Centrifuge all tubes, except "Total count" tubes in a refrigerated centrifuge at approximately 2,500 x g for 30 minutes.
 12. Aspirate the supernatant carefully.
 13. Count the radioactivity of all tubes for 1 minute.

ASSAY SCHEME

Tubes	Total Activity	NSB	CAL0	CAL	Controls	Samples
Reagent						
NSB	-	50 µl	-	-	-	-
Calibrator 0	-	50 µl	50 µl	-	-	-
Calibrators*	-	-	-	50 µl	-	-
Controls	-	-	-	-	50 µl	-
Samples	-	-	-	-	-	50 µl
TSH	-	-	-	-	-	50 µl
Receptor	-	-	50 µl	50 µl	50 µl	50 µl

- Mix gently and incubate: 10 min. R.T. shaking (150 rpm)

Tracer	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
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- Incubate : 2 h. R.T. shaking (150 rpm)

PEG	-	2 ml	2 ml	2 ml	2 ml	2 ml
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- Centrifuge : 30 min. (4°C) at 2,500 g

- Aspirate the supernatant.

- Count the radioactivity of tubes for one minute in a gamma counter.

* only for method with calibrators.

8. CALCULATION OF THE RESULTS

- assay validation

- Determine the average count rate (c.p.m.) for each set of duplicate tubes.
- Evaluate the percentage of **NON SPECIFIC BINDING (NSB)** as follows:

$$\frac{\text{NSB}}{\text{T}} (\%) = \frac{\text{NON SPECIFIC BINDING mean count rate}}{\text{TOTAL mean count rate}} \times 100$$

- Evaluate the **MAXIMUM BINDING RATE** used in the test as follows:

$$\frac{B_0 - \text{NSB}}{\text{T}} (\%) = \frac{\text{CALIBRATOR 0 mean count rate} - \text{NSB mean count rate}}{\text{TOTAL mean count rate}} \times 100$$

- with calibrators

First, calculate for each duplicate the average NSB-corrected counts :

Net tube counts = Average tube counts minus Average NSB counts

Calculate the binding of each duplicate as a percent of the binding of labelled TSH to TSH receptors in the presence of negative control serum (B₀) :

$$\text{Percent Bound} = \frac{\text{Net Counts}}{\text{Net } B_0 \text{ Counts}} \times 100$$

Using Logit-Log paper, plot Percent Bound on the Y-axis against Concentration on the X-axis for each calibrator. Draw a straight line through the calibration points. Concentrations for the unknowns are then estimated from the line by interpolation.

In case of computer-assisted analysis, a special program suitable for radioimmunoassays and adapted to the counter-computer system should be used.

Example of calculation WITH CALIBRATORS

The values reported below must be considered as an example and may not be used in place of experimental data.

Description	Average cpm.	B/B0 (%)	TRAB conc. (U/l)
Total Activity (T)	20230	-	-
NSB	832	-	-
CAL 0	8941	100	0
CAL 1	8229	91.2	5
CAL 2	6011	63.9	15
CAL 3	3999	39.1	45
CAL 4	2441	19.8	135
CAL 5	1725	11.0	405
CONTROL 1	6005	63.8	15.9
CONTROL 2	4231	41.9	44.3
P1	7836	86.4	5.7
P2	3679	35.1	53.7
P3	2675	22.7	109.5

- without calibrators

The reference points for the assay are determined using the calibrator 0 supplied. First subtract the control receptors (NSB) from each tubes to obtain specific TSH binding. Specific bound = Tube count minus NSB

Then, calculate inhibition of TSH binding as follow :

$$\text{Inhibition } (\%) = \left(1 - \frac{\text{Specific bound in the presence of test sample}}{\text{Specific bound in the presence of calibrator 0}} \right) \times 100$$

Example of calculation WITHOUT CALIBRATORS

The values reported below must be considered as an example and may not be used in place of experimental data.

Description	Average cpm.	Inh (%)
Total Activity (T)	20230	-
NSB	832	-
CAL 0	8941	-
CONTROL 1	6005	36.2
CONTROL 2	4231	58.1
P1	7836	13.6
P2	3879	64.9
P3	2675	77.3

9. REFERENCES VALUES

It is recommended that each laboratory determines its own reference interval. Values reported below are only indicative.

- with calibrators

Lower than 9 U/l:	Negative for TSH-R auto-antibodies.
Between 9–14 U/l	Borderline
Higher than 14 U/l:	Positive for TSH-R auto-antibodies.

- without calibrators

When working without calibrators for inhibition values to consider positive results, please refer to quality control sheet included in the kit.

10. PERFORMANCE OF THE ASSAY

SPECIFICITY

Addition to the following levels of possible interfering substances caused no significant difference in quantitative TSH RECEPTOR AUTO-ANTIBODIES levels.

Substances	Concentrations
FSH	≤23000mIU/ml
LH	≤1200mIU/ml
HCG	≤140ng/ml
Thyroglobulin	≤10µg/ml
Prolactin	≤135ng/ml
TSH	≤890mIU/l
HGH	≤1000ng/ml
IgM*	≤4.39mg/ml

IgA*	≤4.23mg/ml
IgG*	≤36mg/ml

* total concentration (serum concentration + immunoglobulins added)

SENSITIVITY

Analytical sensitivity

The sensitivity was calculated based upon the calibration curve and expressed as the minimal dose showing a significant difference from the Zero Calibrator (mean value - 2 S.D.). This dose is 0.8 U/l.

Functional sensitivity

The functional assay sensitivity is the lowest value which is measured with a precision of max. 20% inter-assay variance. For the TSH-R antibodies, this value is 5.0 U/l.

PRECISION

Precision was evaluated upon intra- and inter-assay variability at different analyte concentrations.

Intra-assay

Serum	Mean	± (U/l)	S.D.	C.V. (%)	N
1	18.4	±	1.4	7.6	20
2	29.8	±	2.3	7.7	20
3	79.3	±	7.7	9.7	20

Inter-assay

Serum	Mean	± (U/l)	S.D.	C.V. (%)	N
1	17.3	±	1.7	9.8	9
2	52.5	±	2.7	5.1	9
3	93.4	±	8.6	9.2	9

ACCURACY

Accuracy of the method has been checked by the recovery and parallelism test

Recovery test: Samples, mixed with equal volumes of each calibration point, were tested.

	MEASURED (U/l)	EXPECTED (U/l)	RECOVERY (%)
S1	15	-	-
S1 + CAL 0	4	8	59.1
S1 + CAL 1	9	10	90.7
S1 + CAL 2	16	15	105.3
S1 + CAL 3	28	30	93.4
S1 + CAL 4	78	75	103.4
S1 + CAL 5	244	210	116.0
S2	76	-	-
S2 + CAL 0	45	38	117.5
S2 + CAL 1	53	41	129.3
S2 + CAL 2	52	46	114.5
S2 + CAL 3	73	61	119.5
S2 + CAL 4	135	106	127.3
S2 + CAL 5	283	241	117.7

Parallelism test: Samples with high analyte concentrations were tested at different dilution with the zero calibrator.

DILUTION	MEASURED (U/l)	EXPECTED (U/l)	RECOVERY (%)
S1 undiluted	210	-	-
1/2	123	108	113.9
1/4	63	54	116.7
1/8	27	27	100.0
S2 undiluted	139	-	-
1/2	71	69.5	102.2
1/4	37	34.7	106.6
1/8	16	17.4	92.0

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