

Direct ^{125}I -Immunoradiometric assay kit for the quantitative determination of serum anti-thyroid peroxidase auto-antibodies.

Cat. BL-07-100
040702 - Rev. 10

Summary and background of the test:

Immunological action on the cells of the thyroid gland can either result in stimulating or suppressing thyroid activity (1). Auto-antibodies to several distinct thyroid antigens, including thyroglobulin (Tg), thyroid microsomal antigen, and the TSH receptor, are often present in the sera of patients with hyperthyroid Graves' disease. This disorder may include a peculiar condition known as exophthalmos. Thyroid hormones are produced in excess (2).

In cases of Hashimoto's goitre, antibodies to thyroglobulin are synthesized by immunocytes invading the thyroid gland, diminishing its function that may disappear entirely, if untreated. Similarly, patients with autoimmune hypothyroidism often have serum antibodies to thyroid microsomal antigen (3), identified as thyroid peroxidase (TPO) (4 → 13).

The most widely used method for the detection of circulating auto-antibodies has been passive haemagglutination. However, this technique allows only semi-quantitative determination, and its sensitivity may be inadequate for special investigation purposes.

Different techniques are presently used to detect and quantify auto-antibodies to thyroglobulin and thyroid peroxidase : antigen-coated plates (14), antigen-coated tubes (15), monoclonal antibody-coated tubes (16), and direct binding of ^{125}I -labelled antigens to corresponding auto-antibodies (17).

The present assay system is based on the direct interaction between ^{125}I -labelled Thyroid peroxidase (microsomal Ag.) and auto-antibodies.

Both design of the kit and standardization against MRC preparation 66/387 for microsomal antibodies result in improved sensitivity, precision, and ease of handling.

Principle of the test :

If present, anti-thyroid peroxidase auto-antibodies from the samples will bind ^{125}I -labelled human TPO. Simultaneously, IgG-h-TPO complexes will be precipitated by Protein A, exhibiting a strong affinity for the Fc-chain of most mammalian

immunoglobulins and their antigen complexes.

The activity of the precipitate will be directly related to the levels of the anti-TPO antibodies initially present.

The level of unknown auto-antibodies is then determined by comparing the radioactivity of the isolated precipitate with data established using known standards in the same assay system.

Results are expressed in IU/ml, calibrated on the international reference preparation of human anti-microsomal serum (MRC 66/387).

Precautions:

1. Radioactive material: Radioactive material may be received, acquired, possessed and used only by physicians, clinical laboratories, or hospitals for "In-Vitro" clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals.

Compliance with these basic rules of radiation safety should provide adequate protection:

1. Do not eat, drink, smoke, or apply cosmetics in areas where radioactive material is used.
2. Do not pipet by mouth reagents containing radioactive materials.
3. Wear protective clothing; i.e., lab coats and disposable gloves, in order to avoid direct contact with radioactive reagents.
4. Work with radioactive materials should be performed in a designed area.
5. Radioactive materials should be stored in an acceptable location.
6. A log should be kept for receipt and disposal of radioactive materials.
7. Radioactive spills or accidents should be taken care of immediately according to established procedures.
8. Disposal of radioactive materials must comply with prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory.

2. Sodium azide: Sodium Azide, used as a bacteriostatic agent, is toxic in acid medium. In addition, it may form potentially explosive lead or copper azides. To avoid dangerous deposits, waste solutions should be flushed away with large volumes of water.

3. Hepatitis and Acquired Immune Deficiency Syndrome (HTLV-III): All Bio-Line reagents included in this kit have been tested and found to be non reactive for hepatitis B surface antigen. They have also been screened and determined to be non-reactive for HTLV-III antibody. However, human serum products should be handled as if potentially capable of transmitting hepatitis, Acquired Immune Deficiency Syndrome, or other infectious agents.

Materials provided

Kit contains sufficient reagents for 100 determinations.

1. **Anti-TPO Ab standards & control** : 7 vials each containing 500 μ l. Control: 150 \pm 25 IU/ml.
Standards: 0-50-150-500-1500-5000 IU/ml.
2. **¹²⁵I-TPO tracer** : 2 vials (yellow solution) containing each 5.2 ml. Ready for use.
Activity per vial \leq 3 μ Ci or \leq 111 kBq.
3. **Protein A** : 2 vials containing each 10.5 ml ready-to-use blue suspension (Staphylococcus aureus cells).
4. **Diluent** : 1 vial containing 60 ml of ready-to-use blue-colored solution.

All reagents are ready for use and should be stored at 2° - 8° C.
Refer to the expiration date on the kit label for stability.

Materials required but not provided:

1. Pipets, micropipets, repeating syringes and repeating pipettors.
2. Plastic disposable tubes.
3. Cooling centrifuge capable of developing 1300 to 1500g.
4. Gamma counter.
5. Logit log graph paper.
6. Test tube racks.
7. Vortex mixer.
8. Magnetic stirrer and stir bars.
9. 9‰ NaCl saline solution.

Specimen collection and preparation:

Sera should be separated from blood cells immediately after collection. Sera are stable for at least 7 days at 4° C and for longer periods of time when stored frozen.

Pre-dilution of patient samples:

Before analysis, samples must be diluted 1/20 in the diluent as follows:

25 μ l samples + 500 μ l Diluent (blue).

Do not attempt to dilute the standards and control serum ; they are already diluted and ready for use.

Assay procedure:

Bring reagents to room temperature and mix before use. Label normal or conical polystyrene disposable tubes for total counts (Tc), standards, control sera and unknowns.

1. Pipet 50 μ l of standards and control, 1/20 pre-diluted samples into the corresponding tubes.
2. Add 100 μ l tracer solution (yellow) to each tube.
3. Mix and incubate for 1 hour at room temperature.
4. Add 200 μ l of Protein A to each tube, except Tc. Maintain moderate magnetic stirring of the suspension during the transfer.
5. Mix and incubate for 10-15 minutes at room temperature.
6. Add 2 ml of NaCl 9‰ to each tube, except Tc. Centrifuge all tubes, except Tc, for 10-15 minutes at 1300-1500g at 4°C.
7. Decant all tubes as a whole, discarding the supernatant into a radioactive waste container. While tubes are inverted, gently blot the final drops onto absorbent paper. Do not aspirate. Do not let stand inverted.
When using **conical tubes**, an aspiration of the supernatant should be possible, using a suction device with the needle adjusted so that it is just 2 mm above the visible precipitate.
8. Record the counts per minute (cpm) for each tube.
Count all tubes for one minute

TPO Flow chart.

Tubes Reagents	Tc	Standards	control	1/20diluted samples
Standards /samples	-	50 µl	50 µl	50 µl
Tracer	100 µl	100 µl	100 µl	100 µl
Mix and incubate at room temperature for 1 hour				
Protein A	-	200 µl	200 µl	200 µl
Mix and incubate at room temperature for 10-15 minutes				
NaCl 9‰	-	2 ml	2 ml	2 ml
Centrifuge 15 minutes at 4°C and discard supernatants				
Count in a gamma counter				

Data table (Example).

#	Duplicate cpm		Mean cpm	%B/Tc	Conc. IU/ml
Tc	64 666	66 489	65 578	100 %	
Zero	1 911	1 910	1 911	2.9 %	
Std 50	7 001	7 141	7 071	10.8 %	
Std 150	11 087	10 834	10 961	16.7 %	
Std 500	15 447	15 834	15 641	23.9 %	
Std 1500	22 979	23 339	23 159	35.3 %	
Std 5000	34 851	34 505	34 678	52.9 %	
Control	10 737	10 701	10 719	16.3 %	152
Sample 1	22 489	22 385	22 437	34.2 %	1395

Calculation of results:

Data need not be expressed as counts per minute (cpm) but the counting period must be the same for all tubes that are counted.

Determine the average counts for each set of duplicate tubes. Divide this value by the average net counts of Tc, and multiply by 100 to yield the % B/Tc.

$$\% \text{ B/Tc} = \frac{\text{cpm (Stds, Control or samples)}}{\text{cpm (Tc)}} \times 100$$

Plot % B/Tc for each standard vs its concentration in IU/ml. The concentration of anti-TPO in the unknown samples may be read directly from the standard curve.

Samples exhibiting titers greater than 5000 IU/ml should be more diluted using the diluent. If necessary, multiply by the dilution factor to determine the initial concentration.

Expected Values:

Each laboratory should establish its own normal range.

Anti-TPO values
 ≤ 100 IU/ml
 100-200 IU/ml
 ≥ 200 IU/ml

Interpretation
 Negative
 Gray zone
 Positive

Bibliography:

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