



INSTRUCTION MANUAL

IVD

(July 21st, 2005)

SELco[®] anti-TPO

- 100 determinations -

REF 1500



Radioimmunoassay for the determination of autoantibodies to Thyroid Peroxidase (TPO) in human serum



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INTENDED USE

SELco[®] anti-TPO is used for the quantitative and highly sensitive determination of thyroid peroxidase autoantibodies (anti-TPO) in human serum. TPO itself is a membrane-bound enzyme in the thyroid cell. It is essential for the thyroid hormone synthesis and is one of three thyroid antigens.

One of the characteristics of autoimmune thyroid diseases is the presence of autoantibodies to thyroid antigens.

Consequently, the anti-TPO determination is indicated for the detection of chronic autoimmune thyroiditis and for the differential diagnosis of hypothyroidism including its subclinical or latent type. The anti-TPO determination is also valuable in Graves' disease, particularly if the TSH Receptor Antibody value is negative, but the disease is clinically suspected. Another indication is the exclusion of a co-existing thyroid autoimmune disease in case of euthyroid goiter.

PRINCIPLE of the TEST

SELco[®] anti-TPO is a competitive one-step radioimmunoassay using coated tube technique. Monoclonal antibodies to human thyroid peroxidase are coated to the inner side of the tube and compete with the autoantibodies of the patients sample for similar epitopes of ¹²⁵I-labelled recombinant human thyroid peroxidase provided as tracer. Following an incubation step of 90 minutes unbound tracer, as well as the autoantibody-tracer complexes in the liquid phase are removed by a dilution step and decantation. The remaining radioactive signal of the tube is measured in a gamma-counter.

Maximum binding is generated if autoantibodies against TPO are absent in the sample, as there is no competing reaction and the coated monoclonal antibodies of the tube will be saturated by acquiring the provided TPO tracer. Increasing amounts of autoantibodies in the sample intense competition thus catching more of the provided ¹²⁵I-labelled TPO tracer and consequently decreases the radioactive signal of the tubes.

Hence the measured radioactivity (cpm) is inverse proportional to the autoantibody concentration, which may be read off directly against a standard curve, established either with cpm values or with binding values B/B₀%.

The unique assay design of SELco[®] anti-TPO spares both, the cumbersome dilution of the patient samples as well as it obtain accurate results with an incubation time of only 90 minutes.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. Lipaemic and hemolytic samples should not be employed.

The samples may be kept at 2 - 8 °C up to three days. Long-term storage requires - 20 °C.

Repeated freezing and thawing should be avoided. For multiple use, initially aliquot samples and keep at - 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: Patient samples have not to be diluted. Use native serum.

IFU symbols radioactive assays MEDIPAN GMBH



In vitro diagnostic device



EC Declaration of Conformity



Catalogue number



Batch code



Expiry date



Manufactured by



Consult accompanying documents



Consult operating instruction



Store at



Biological risk



Radioactive component



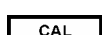
Coated tube



Wash buffer



Tracer



Calibrators



Control serum



Buffer

TEST COMPONENTS for 100 DETERMINATIONS

A **Coated tubes** **2 x 50 tubes**
ready for use
CT coated with human monoclonal antibodies
 Allow tubes to reach room temperature within the package, prior to use.

D1 **Tracer, 125-I-TPO, human** **2 bottles**
lyophilized
TRAC < 41 kBq, each
 for reconstitution with 14 ml of BUFD

Reconstitute the content of D1 with approx. 5 ml of BUFD, each, and allow 10 min. of solving. Then transfer this solution completely into the rest of BUFD and **re-label the bottle of BUFD with the provided labels D2 accordingly.**

D2 **Labels for reconstituted tracer** **2 labels**
TRAC The reconstituted tracer remains stable for one month at 2 - 8 °C within the stated shelf live of the kit.

J **Buffer** **2 bottles,**
14 ml each,
ready for use
BUF D for reconstitution of tracer D1

Note: This buffer bottle serves for the reconstituted tracer and should be re-labeled with the provided label D2 consequently.

B **Wash buffer** **1 bottle,**
20 ml
WASHB (Concentrate for 500 ml)

 Add **480 ml** distilled water to a final volume of 500 ml.

0 **Zero Calibrator** **1 vial**
ready for use
CAL 0.2 ml serum

1 - 5 **anti-TPO Calibrators** **5 vials**
0.2 ml each
ready for use
CAL (serum)
Conc.: 8; 40; 200; 1000; 7500 U/ml

CI - C II **anti-TPO Control Sera** **2 vials**
0.2 ml each
ready for use
CONTROL (serum)
Conc.: cf. leaflet enclosed

Size and storage

SELco® anti-TPO has been designed for 100 determinations. This is sufficient for the analysis of 42 unknown samples as well as for calibrators and control sera, assayed in duplicates.

The expiry date of each component is reported on its respective label that of the complete kits (max. 7 - 8 weeks) on the box label.

Upon receipt, all components of the SELco® anti-TPO have to be kept at 2 - 8 °C, preferably in the original kit box.

Preparation before use

Allow all of the components to reach room temperature prior to use in the assay.

- Duplicates are recommended.

1. Label test tubes (**A**) appropriately.
2. Pipette into the corresponding tubes according to assay scheme.
 - 20 µl calibrators,
 - 20 µl control sera,
 - 20 µl **native** patient's samples, each
3. Add **250 µl tracer D2**, to **all tubes**, including those for total radioactivity **T**.
Tubes T are now separated until radioactivity is measured.
4. Incubate for 90 minutes while agitating (at room temperature).
5. Add 2 ml washing solution (made from B), each.
6. Aspirate supernatant completely or decant. For removal of any remaining liquid, turn tubes upside down (5 to 10 minutes) and absorb any droplets by tapping on blotting paper.
7. Measure radioactivity of **all tubes including T**.
 Recommended counting time: 1 minute

DATA PROCESSING

The standard curve is established by plotting the mean cpm-values of the calibrators 0 - 5 on the ordinate, y-axis, (log. scale) versus their respective anti-TPO-concentrations on the abscissa, x-axis, (log. scale).

The anti-TPO concentrations of the controls and the unknown samples are **directly read off** in U/ml against the respective cpm values.

The respective binding rates B/B₀ (%), may be used as well for setting up the standard curve.

SELco® anti-TPO may be used also with Computer Assisted Analysis using software able to plot curves with spline smoothing such as typically for radioimmunoassays.

Any extrapolation of the standard curve with regard to anti-TPO values above 7500 U/ml (calibrator 5) is not permitted. Measurements of anti-TPO concentrations, which are off curve, are possible by diluting these sera once with negative patient serum 1 : 20 (vol / vol).

ASSAY PROCEDURE

TYPICAL EXAMPLE

(approx. 4 weeks before expiry)

Do not use for evaluation!

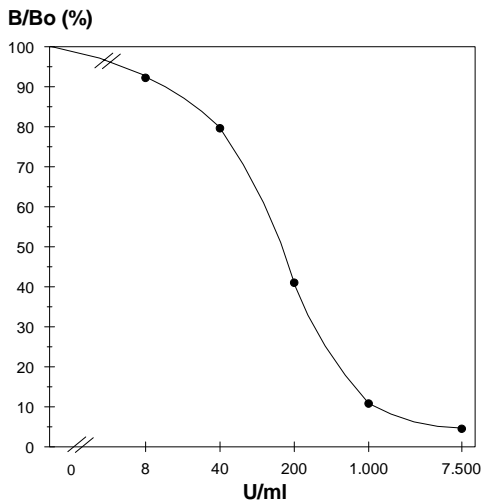
Test tubes	cpm (a)	cpm (b)	cpm (mean)	$\frac{B}{B_0}$ %	U/ml
Total radioactivity	30553	30344	30449	---	---
Calibrator 0	16995	17415	17205	100	0
Calibrator 1	16042	15672	15857	92.2	8
Calibrator 2	13540	13860	13700	79.6	40
Calibrator 3	6864	7274	7069	41.0	200
Calibrator 4	1944	1784	1864	10.8	1000
Calibrator 5	736	818	777	4.5	7500
Control I		cf. leaflet enclosed			
Control II		cf. leaflet enclosed			
Patient 1	5379	5486	5433	31.6	290

Calculation of patient sample 1:

$$\frac{B}{B_0} (\%) = \frac{5433}{17205} \times 100 = 31.6$$

STANDARD CURVE

Typical example



REFERENCE VALUES

SELco® anti-TPO	U/ml
negative	≤ 40
grey zone	40 - 50
positive	> 50

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

More than 90 % of the cases of autoimmune thyroiditis are anti-TPO positive. In active Graves' disease the proportion of anti-TPO positive results is above 70 %.

Subacute thyroiditis De Quervain normally results as anti-TPO negative. Even low positive values of anti-TPO indicate that autoimmune processes occur. However, this does not inevitably mean that any thyroidal dysfunction already exists or is clinically manifest.

CHARACTERISTIC ASSAY DATA

Binding capacity

The maximum binding capacity of the SELco® anti-TPO is defined by means of the zero calibrator (B_0/T %) and is usually found between 50 % and 75 %.

$$\frac{B_0}{T} (\%) = \frac{\text{mean cpm } B_0}{\text{mean cpm } T} \times 100$$

Calibration

The calibration of SELco® anti-TPO is orientated towards NIBSC-reference serum 66/387, which was established at that time for the conventional determination of microsomal antibodies (NIBSC: Nat. Inst. for Biol. Standards and Control, Hampstead, London, U.K.).

Parallelism of serum samples

Due to the inherent heterogeneous nature of the autoantibody population in human sera with regard to epitopes and affinity, dilutions of patient samples with autoantibody free human serum are not necessarily found congruent to the expected values via calculation in all cases.

Specificity

The specificity of the chosen coated monoclonal antibodies, recognizing defined epitopes similar to those of the autoantibody population in the samples, as well as the high quality of the provided tracer (125-I-hTPO) are essential for a true competition within the reaction. These are also guarantors for the absence of any detectable cross-reactions with auto-antibodies to Thyroglobulin (cf. SELco® anti-Tg) or to TSH receptor (cf. TSH-REZAK®).

Sensitivity (lower detection limit)

The so-called analytical sensitivity of SELco® anti-TPO is approximately 6 U/ml, however, the most appropriate and statistically reasonable definition of the lower detection limit of any assay is at present the so-called **functional assay sensitivity**.

This functional assay sensitivity generally represents that concentration which corresponds to the 10 % (within-assay) and to the 20 % (between assay) coefficient of variation in the respective precision profiles of the assay in the lower concentration range.

Upon correct and thorough performance of SELco® anti-TPO, this value is found at approx. 30 U/ml.

Anti-TPO values below this defined level of functional assay sensitivity do not meet the statistical criteria for reliability according to GLP (Good Laboratory Practice) and therefore can not be distinguished from zero due to the statistically necessary certainty.

LIMITATIONS of the METHOD

Any clinical diagnosis should not be based on the results of in vitro diagnostic method alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.




SELco[®] anti-TPO

ASSAY SCHEME

1	Label test tubes A	CAL 0 - 5	CI - CII	Patients	T
2	Pipette	Calibrators 0 - 5 Controls CI - CII Patient samples (native)	20 µl	20 µl	20 µl
3	Pipette	Tracer D2	250 µl	250 µl	250 µl
4	Incubate	90 minutes agitating at room temperature			
5	Pipette	Washing solution (made from B)	2 ml	2 ml	2 ml
6	Decant or Aspirate	and leave tubes upside down on absorbent paper (for 5 - 10 minutes) quantitatively			
7	Count radioactivity	counting time: 1 minute			

SAFETY PRECAUTIONS

This kit is for in vitro use only. Follow the working instructions carefully.

- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
-  Some of the reagents contain small amounts (< 0.1 % w/w) of sodium azide as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa. The possible formation of heavy metal azides in the drainage has to be prevented by sufficient rinsing with water.
-  Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for both Hepatitis and HIV antibody. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
-  Since the kit contains radioactive material the following precautions should be observed:
 - Do not smoke, eat or drink while handling radioactive material,
 - Always use protective gloves,
 - Never pipette radioactive material by mouth,
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant,
 - Place contaminated tissues, tubes, bench covers, gloves etc. in a specially marked container, discard liquid and solid radioactive waste only as permitted by federal, state or local authorities and regulations.
- It is the responsibility of the user of this product to handle radioactive material in accordance to the national rules given by law or other statements of the local authorities.
- In any case GLP should be applied with all general and individual regulation to the use of this kit.