



# Human Thyroid Peroxidase (TPO) autoantibodies ELISA Kit

Enzyme Immunoassay for the quantitative screening of IgG autoantibodies to Thyroid Peroxidase in serum or plasma.

Catalog number: ARG80892

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For research use only. Not for use in diagnostic procedures.

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## Human TPO autoantibodies ELISA Kit ARG80892

### PRINCIPLE OF THE ASSAY

This assay employs the qualitative enzyme immunoassay technique. Highly purified human thyroid peroxidase (TPO) has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated anti human antibody is added to each well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm  $\pm$ 2 nm. The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

### MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Calibrator A-F (0, 33, 100, 330, 1000, 3000 IU/ml)	6 X 1.5 ml (Ready-to-use)	4°C.
Control Positive	1.5 ml (Ready-to-use)	4°C.
Control Negative	1.5 ml (Ready-to-use)	4°C.
5X Sample Buffer	20 ml	4°C

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HRP-Antibody conjugate	15 ml (Ready-to-use)	4°C
50X Wash buffer	20 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C

### MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

### TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-antibody conjugate before use.
- If crystals are observed in the 50X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

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### SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Serum**- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

### REAGENT PREPARATION

- **1X Wash buffer:** Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer.
- **1X Sample buffer:** Dilute 5X Sample buffer into distilled water to yield 1X Sample buffer.
- **Patient sample:** Dilute patient sample 1:100 with sample buffer before assay.

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### ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100  $\mu$ l of diluted samples, Calibrators and controls into wells. Leave one well empty for the substrate blank.
3. Incubate for 60 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1 $\times$  Wash Buffer (350  $\mu$ l) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100  $\mu$ l HRP-antibody conjugate into each well (except the substrate blank). Incubate for 15 minutes at RT.
6. Wash as according to step 4.
7. Add 100  $\mu$ l of TMB Reagent to each well. Incubate for 15 minutes at room temperature.
8. Add 100  $\mu$ l of Stop Solution to each well. Incubate for 5 minutes at RT.
9. Read the OD with a microplate reader at 450 nm immediately.

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### **CALCULATION OF RESULTS**

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may be estimated from the calibration curve by interpolation.

Using data reduction software a 4-parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

### **INTERPRETATION OF RESULTS**

Negative: < 50 IU/ml

Equivocal: 50-75 IU/ml

Positive: > 75 U/ml

### **QUALITY ASSURANCE**

#### **Limit of detection**

Functional sensitivity was determined to be 5 IU/ml.

#### **Interferences**

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparin). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

#### **Intra-assay and Inter-assay precision**

The CV value of intra-assay precision was 2% and inter-assay precision was 5.43%.