Human Parietal Cell antibody ELISA Kit

Enzyme Immunoassay for the quantification of human IgG antibodies to Parietal Cell H+/K+ ATPase in serum or plasma.

Catalog number: ARG80401

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

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INTRODUCTION

Circulating autoantibodies to gastric parietal cells have been first detected in patients with pernicious anemia by the complement fixation test, described by Irvine et al. 1962 and following with an immunofluorescence test described by Taylor et al.1962. The responsible parietal cell autoantigen was localized to the secretory canaliculi of gastric parietal cells and to gastric microsomes. Further biochemical and molecular investigations identified the responsible antigens as alpha- and beta-subunit of the gastric H+/K+-ATPase.

The gastric H+/K+-ATPase (EC 3.6.1.3) is a hydrogen transporting enzyme, responsible for the acidification of the stomach lumen (Rabon and Reuben, 1990). It belongs to the family of electroneutral Ptype ATPases which also include the Na/K and the Ca ATPases (Pederson and Carfoli, 1987). This parietal cell antigen consists of two subunits, an 8-10 transmembrane catalytic alpha-subunit of 1033 amino acids and a heavily glycosylated beta-subunit with a 294 amino acid core. This H+/K+-ATPase shows a high degree of conservation in the amino acid sequence a cross species (van Driel and Callaghan, 1995).

Pernicious anemia is the most common cause of vitamin B12 deficiency in Western populations. Longitudinal studies suggest, that pernicious anemia is the end stage of type A chronic atrophic gastritis (Irvine et al. 1974), a disease characterised by pathological lesions of the fundus and body of the stomach, including gastric mucosal atrophy, selective loss of parietal and chief cells from the gastric mucosa and submucosal lymphocytic infiltrates (Whittingham and

Macckay, 1985).

Pernicious anemia is predominately a disease of middle age northern white Europeans and females show a higher incidence than males. Patients with pernicious anemia appear pale, physically tired and mentally depressed. Pernicious anemia associates with a number of other diseases and these are predominantly organ specific autoimmune diseases of endocrine glands, in which autoantibodies to other tissue specific antigens are also present. The specific diseases include Hashimoto's thyroiditis, diabetes mellitus Type 1 and primary Addison's disease (Whittingham and Macckay, 1985). Late stages of pernicious anemia may also be associated with peripheral neuropathy and subacute combined degeneration of the spinal cord due to vitamin B12 deficiency. Autoantibodies against the H+/K+-ATPase can be detected in 80-90% of pernicious anemia patients, by indirect immunofluorescence and they are also detected in 2-5% of the healthy adult population. ELISA test systems show a sensitivity of about 80% and specificity of about 90%. There is an age related increase in the presence of parietal cell autoantibodies in the adult population. A study of the relationship between parietal cell autoantibody and gastric mucosal morphology indicates these parietal cell positive individuals in a random population may indeed have early type A gastritis (Uibo et al., 1984). Higher prevalence rates (20-30%) of parietal cell autoantibodies have been noted in patients with autoimmune endocrine disorders such as thyrotoxicosis, Hashimoto's thyroiditis and insulin dependent diabetes (Whittingham and Macckay, 1985). Histological examinations of gastric biopsies reveal that the majority of parietal cell autoantibody positive individuals also have a type A

gastric lesion (Varis et al. 1979).

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A highly purified pig parietal cell H+/K+ ATPase 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, an HRP-conjugated human antibody is added to each well and incubate. 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 450nm ±2nm.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.
Standard A-F (0, 6.3, 12.5, 25, 50, 100 U/ml)	1 vial, 1.5 ml (Ready-to-use)	4°C
Control 1 (30 U/ml, accept range 20-40 U/ml)	1 vial, 1.5 ml (Ready-to-use)	4°C
Control 2 (2 U/ml, accept range <5 U/ml)	1 vial, 1.5 ml (Ready-to-use)	4°C
5X Sample buffer	20 ml	4°C
HRP-Antibody conjugate	15 ml (Ready-to-use)	4°C
50X Wash buffer	20 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620 nm as reference wavelength)
- 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 in dark at all times.
- 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.
- Prepare all reagents and samples. Once started, perform the test without interruption.
- 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.

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 ≤ -20 °C up to 6 months. 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 ≤ -20 °C up to 6 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. Diluted Wash Solution are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.
- 1X Sample buffer: Dilute 5X Sample buffer with distilled buffer before use. Diluted Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.
- Patient sample: Dilute patient sample 1:100 with 1X sample buffer before assay, mix well. (e.g. 10 µl of sample + 990 µl of 1X sample buffer)
  Note: the controls and calibrators are ready-to-use and need not further dilution.

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 100µl of standards, controls and diluted samples into wells, mix thoroughly.
3. Incubate for 30 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× Wash Buffer (300µl) 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 100µl 1X Antibody solution into each well, mix thoroughly. Incubate for 15 minutes at RT.
6. Wash as according to step 4.
7. Add 100µl of TMB substrate to each well, mix thoroughly. Incubate for 15 minutes at room temperature.
8. Add 100µl of Stop Solution to each well. Incubate for 5 minutes at RT. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm and 600-690 nm as reference wavelength immediately. It is recommended read the OD within 30 minutes after adding Stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

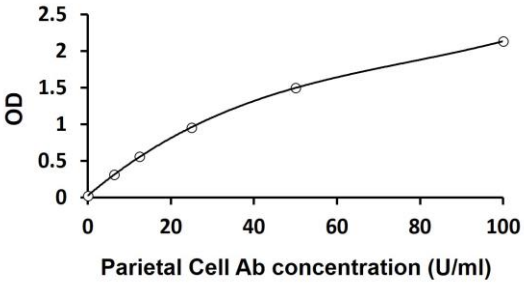
2. Using Log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



INTERPRETATION OF RESULTS

Negative: < 10 U/ml

Positive: ≥ 10 U/ml

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Insulin Ab ranged from 6.3-100 U/ml. The mean MDD was 0.5 U/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.1% and inter-assay precision was 3.5%.