Parietal Cell Ab ELISA Kit

Catalog Number KA1108

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for quantitative determination of IgG Autoantibodies to Parietal Cell H+/K+-ATPase in human serum or plasma

Background

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 localised 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 P-type 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

Highly purified pig parietal cell H+/K+-ATPase is bound to microwells. Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

General Information

Materials Supplied

List of component

Component	Amount
Divisible Microplate: Consisting of 12 modules of 8 wells each, ready to use	96 (8x12) wells
Calibrator A-F (0; 6.3; 12.5; 25; 50; 100 U/mL): containing serum/buffer matrix (PBS, BSA, NaN_3 0.09%), yellow. Ready to use.	1.5 mL x 6
Control positive (1) (32 U/mL) and negative (2) (3 U/mL): Containing parietal cell antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.	1.5 mL x 2
Sample Buffer P: Containing PBS, BSA, detergent, NaN_3 0.09%, yellow, concentrate (5x).	20 mL
Enzyme Conjugate: Containing anti-human-IgG, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready for use.	15 mL
TMB Substrate: Containing 3,3',5,5'- tetramethylbenzidin, colorless. Ready to use.	15 mL
Stop solution: Containing acid. Ready for use.	15 mL
Wash Solution: Containing Tris, detergent, preservative NaN_3 0.09%; 50x conc.	20 mL

Storage Instruction

- ✓ Store the kit at 2-8°C in the dark.
- ✓ Do not expose test reagents to heat, sun or strong light during storage and usage.
- ✓ Store microplate sealed and dessicated in the clip bag provided.
- ✓ Unpoened reagents are stable until expiration of the kit. See labels for individual batch.
- ✓ Diluted Wash solution and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

Materials Required but Not Supplied

- ✓ Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm.
- ✓ Data reduction software
- ✓ Multi-Channel Dispenser or repeatable pipet for 100 µL
- ✓ Vortex mixer
- ✓ Pipets for 10 µL, 100 µL and 1000 µL
- ✓ Laboratory timing device
- ✓ Distilled or deionized water
- ✓ Measuring cylinder for 100 and 1000 mL
- ✓ Plastic container for storage of the wash solution
- ✓ This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be

validated on the respective automated system. Detailed information is provided upon request.

Precautions for Use

- ✓ Procedural Notes
- ✓ Do not use kit components beyond their expiration dates.
- ✓ Do not interchange kit components from different lots and products.
- ✓ All materials must be at room temperature (20-28°C) prior to use.
- ✓ Prepare all reagents and samples. Once started, perform the test without interruption.
- ✓ Double determination may be done. By this means pipetting errors may become obvious.
- ✓ Perform the assay steps only in the order indicated.
- ✓ Always use fresh sample dilutions.
- ✓ Pipette all reagents and samples into the bottom of the wells.
- ✓ To avoid carryover contamination, change the pipette tip between samples and different kit controls.
- ✓ Wash microwells thoroughly and remove the last droplets of wash solution.
- ✓ All incubation steps must be accurately timed.
- ✓ Do not re-use microplate wells.

- ✓ Warnings and Precautions
- ✓ All reagents of this kit are strictly intended for professional use only.
- ✓ Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 and HIV2 and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- ✓ Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- ✓ Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine).
- ✓ Stop Solution contains acid, classification is non-hazardous. Avoid contact with skin.
- ✓ Controls, Calibrator, Sample buffer and wash solution contain Sodium Azide (NaN_3) 0.09% as preservative. This concentration is classified as non-hazardous.
- ✓ Enzyme conjugate contains proClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
- ✓ During handling of all reagents, control and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:
- ✓ First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for a least 10 minutes. Get medical attention if necessary.
- ✓ Personal precautions, protective equipment and emergency procedures:
- Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are

handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls/personal protection: Wear protective gloves of nitrile rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or reginal legislation has to be observed.
- Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

Assay Protocol

Reagent Preparation

✓ Wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 mL prior to use.

✓ Sample buffer P

Prior to use dilute the contents (20 mL) of one vial of sample buffer 5x concentrate with diltilled or deionized water to a final volume of 100 mL.

Sample Preparation

✓ Specimen Collection, Storage and Handling

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20°C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

✓ Preparation of Samples

Dilute all samples 1:100 with sample buffer before assay: Put 990 µL of prediluted sample buffer in a polystyrene tube and 10 µL of sample. Mix well.

Note: Calibrators/Controls are ready to use and need not be diluted.

Assay Procedure

1. Prepare enough microplate modules for all calibrators/controls and samples.
2. Pipet 100 µL of calibrators, controls and prediluted samples in duplicate into the wells.
3. Incubate for 30 minutes at room temperature (20-28°C).
4. Discard the contents of the microwells and wash 3 times with 300 µL of wash solution.
5. Dispense 100 µL of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µL of wash solution.
8. Dispense 100 µL of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add 100 µL of stop solution to each well of the modules.
11. Incubate for 5 minutes at room temperature.
12. Read the optical density at 450 nm (reference 600-690 nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Data Analysis

Calculation of Results

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration cure. The concentration of samples may then be estimated from the calibration cure 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.

Performance Characteristics

✓ Calibration

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

✓ Measuring range

The calculation range of this ELISA assay is 0 - 100 U/mL

✓ Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 10 U/mL

✓ Interpretation of results

Negative: < 10 U/mL

Positive: ≧ 10 U/mL

✓ Linearity

Samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper/lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/mL	Expected U/mL	O/E [%]
1	1:100	91.2	91.2	100
	1:200	45.9	45.6	101
	1:400	21.9	22.8	96
	1:800	11.1	11.4	97
2	1:100	68.4	68.4	100
	1:200	33.7	34.2	99
	1:400	16.8	17.1	98
	1:800	8.5	8.6	99

✓ Limit of detection

Functional sensitivity was determined to be: 0.5 U/mL

✓ Interfering Substances

No interference has been observed with haemolytic (up to 1000 mg/dL), 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.

✓ Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample No	Mean [U/mL]	CV (%)
1	12.5	3.5
2	22.5	2.8
3	75.0	3.2

Inter-Assay		
Sample No	Mean [U/mL]	CV (%)
1	12.0	4.2
2	20.5	3.7
3	85.9	2.6

✓ Study results

Study population	n	N Pos	%
Pernicious anemia	85	63	74.1
Normal human sera	100	3	3.0

Sensitivity: 74.1%

Specificity: 97.0%

Overall agreement: 86.5%

Resources

References

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Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Calibrator A	Sample 1										
B	Calibrator B	Sample 2										
C	Calibrator C	Sample 3										
D	Calibrator D											
E	Calibrator E											
F	Calibrator F											
G	Control +											
H	Control -											