Human Intrinsic Factor antibody ELISA Kit

Enzyme Immunoassay for the quantification of IgG antibodies to Intrinsic Factor in serum or plasma.

Catalog number: ARG80396

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 622 1320

Fax: +886 (3) 553 0266

Email: info@arigobio.com

INTRODUCTION

Biermer´s anaemia or pernicious anaemia is the most common cause of vitamin B12 deficiency in Western populations showing the classical features of megaloblastic anemia (i.e. morphologic and functional abnormalities of the blood cells and marrow precursors related to impairment of DNA synthesis) [1, 2, 3]. It is characterised by a gastric mucosal defect that decreases the synthesis of intrinsic factor and the occurrence of autoantibodies to gastric parietal cells and to intrinsic factor. Human intrinsic factor is a glycoprotein that is exclusively produced by gastric parietal cells. It plays an essential role in the absorption and transport of vitamin B12 across the small intestine [4].

Two types of intrinsic factor autoantibodies exist [5]. Type I antibodies block the cobalamin binding site on the intrinsic factor molecule, preventing uptake of the vitamin. Type II antibodies block a different site of the intrinsic factor molecule that is involved in binding of the intrinsic factor-cobalamincomplex to ileal receptors. Both types of antibodies have the same pathological effect, i.e. preventing cobalamin resorption by ileal receptors.

Serum intrinsic factor autoantibodies can be detected in 50 to 70% of pernicious anaemia patients and are highly specific for Biermer´s anemia with no reported single true positive in a healthy control [6]. Intrinsic Factor Ab ELISA detects both types of autoantibodies and thereby provides a useful tool in the differential diagnosis of pernicious anaemia and other causes of vitamin B12 malabsorption.

1. Gleeson, P.A., and B.-H. Toh. Molecular targets in pernicious anaemia. Immun. Today, 1991, 12(7):233-238.

2. Beck, W.S. Neuropsychatric Consequences of Cobalamin Deficiency. Adv. Inter. Med. 1991, 33-56.

3. OH R, Brown D. L. Vitamin B12 deficiency. Am Fam Physician. 2003, 67(5): 979-986.

4. Seetharam, B., S. Bose, and N. Li. Cellular Import of Cobalamin (Vitamin B-12). J. Nutr., 1999, 129:1761-1764.

5. Schade, S.G., P.L. Feick, M.H. Imrie, and R.F. Schilling. In vitro studies on antibodies to intrinsic factor. Clin. Exp. Immunol. 1967, 2:399-413.

6. Carmel, R. Reassessment of the relative prevalence of antibodies to gastric parietal cell and to intrinsic factor in patients with pernicious anaemia: influence of patient age and race. Clin. Exp. Immunol., 1992, 89:74-77.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A highly purified Human Intrinsic Factor 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 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	6 vials (0, 6.3, 12.5, 25, 50, 100 U/ml)(Ready-to-use)	4°C
Controls	2 vials of Positive and Negative Controls (1.5 ml each) (Ready-to-use)	4°C
5X Sample buffer	20ml	4°C
HRP-Antibody conjugate	15ml (Ready-to-use)	4°C
50X Wash buffer	20ml	4°C
TMB substrate	15ml	4°C (Protect from light)
STOP solution	15ml	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 antibody conjugate concentrate and HRP-Streptavidin concentrate 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.

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. 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. 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 with distilled water before use.
- 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, samples and zero controls into wells.
3. Incubate for 30 minutes at RT.
4. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350µl) 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 HRP-antibody conjugate into each well. Incubate for 15 minutes at RT.

6. Wash as according to step 4.
7. Add 100µl of TMB Reagent to each well. 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 immediately.

CALCULATION OF RESULTS

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

2. Using 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.

INTERPRETATION OF RESULTS

Negative: <6U/ml

Positive: >6U/ml

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Intrinsic Ab ranged from 6.3-100 U/ml.

The mean MDD was 0.5 U/ml.

Interference

No interference has been observed with the following factors:

Haemolytic sera/plasma (up to 1000mg/dl)

Lipemic sera/plasma (up to 3g/dl triglycerides)

Bilirubin containing sera/plasma (up to 40mg/dl)

Antiboagulants (Citrate, EDTA, Heparin).

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.6% and inter-assay precision was 4.4%.