AUTOZYME™ IFAB

Anti-Intrinsic Factor Antibodies

REF **Z4396**

Instructions for Use

IVD For in vitro diagnostics use only

Σ **96 Tests**

2°C 8°C **Store at 2 - 8°C**

EC REP

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1. Intended Use

AUTOZYME™ IFAB Anti-Intrinsic Factor Antibodies Assay for the quantitative or semi-quantitative detection of antibodies to Intrinsic Factor (IF) in human serum or plasma to aid the diagnosis of pernicious anaemia in conjunction with other laboratory tests and clinical findings.

2. Background

Pernicious anaemia is the disease most frequently associated with vitamin B12 deficiency. This disease shows the classical features of megaloblastic anaemia (i.e. morphologic and functional abnormalities of the blood cells and marrow precursors related to impairment of DNA synthesis).
Pernicious anaemia is characterised by a gastric mucosal defect that decreases the synthesis of intrinsic factor. Intrinsic factor is a 50kDa glycoprotein secreted by the gastric parietal cells which plays an essential role in the transport and absorption of vitamin B12 across the small intestine, patients affected by pernicious anaemia show severe decreases in serum vitamin B12 levels.
The presence of autoantibodies against gastric parietal cell and intrinsic factor, strongly suggest an autoimmune process to the disease.
There are two types of anti-intrinsic factor antibodies: Type I block the binding of cobalamin to IF and thereby prevent the uptake of vitamin B12 (blocking antibodies); Type II prevent the attachment of IF cobalamin complex to the ileum receptors (binding antibodies). Both these antibodies types (type I and type II) result in the same pathological effect, i.e. preventing vitamin B12 absorption. Detection of anti-intrinsic factor antibodies provides an important contribution to the differential diagnosis of pernicious anaemia (due to intrinsic factor deficiency) and other causes of vitamin B12 malabsorption. Indeed, other tests such as the cytomorphology of red blood cells, determination of serum vitamin B12 levels or the Schilling test are not specific enough for the diagnosis of Pernicious anaemia.
Anti-Intrinsic Factor antibodies can be detected using two types of methodologies: RIA or ELISA methods. The ability of type I auto-antibodies to prevent the binding of vitamin B12 to the intrinsic factor has allowed the development of RIA methods. ELISA methods detect type I and type II antibodies and are unaffected by the presence of high exogenous vitamin B12 levels.
The AUTOZYME™ IFAB Anti-Intrinsic Factor Antibodies Assay is an easy, rapid and sensitive method allowing the detection of total anti-intrinsic factor antibodies. The use of recombinant intrinsic factor as antigen ensures the specificity of the method.

3. Principle

The AUTOZYME™ IFAB Anti-Intrinsic Factor Antibodies Assay is a solid phase immunoassay. The wells are coated with recombinant intrinsic factor antigen followed by blocking the unreacted sites to reduce non-specific binding.

First Incubation
Controls and diluted patient serum samples are incubated in the antigen coated wells which allow specific antibodies present in the serum to bind to the intrinsic factor antigen. The unbound or excess antibodies and other serum proteins are washed off.
Second Incubation
An anti-human IgG coupled peroxidase conjugate is then added to the well and incubated. Any unbound conjugate is washed off.
Third Incubation
Enzyme substrate (TMB) is then added to the wells and the presence of antibodies is detected by a colour change produced by the conversion of TMB substrate to a coloured reaction product. The reaction is stopped with a low pH solution and the intensity of the colour change, which is proportional to the concentration of antibody, is read at 450/620nm dual wavelengths. Results are expressed in arbitrary units per millilitre (AU/ml) and are reported as positive or negative.

4. Kit Contents

SORB	1	Microplate (ready to use) in foil pouch coated with recombinant intrinsic factor. 12 x 8 Break-a-part wells.
CAL	6 x 1.5mL	Calibrators. Human sera ready to use anti-intrinsic factor level 0 AU/ml (**1**), 12.5 AU/ml (**2**), 25 AU/ml (**3**), 50 AU/ml (**4**), 100 AU/ml (**5**), 200 AU/ml (**6**). Calibrator 2 = Cut-off Calibrator (yellow cap) , NHS/PBS/NaN_3 (<0.1%w/v)).
CONTROL −	1 x 1.5ml	Negative Control (red cap), ready to use (yellow solution, NHS/PBS/NaN_3 (<0.1%w/v)).
CONTROL +	1 x 1.5ml	Positive Control (green cap), ready to use (yellow solution, NHS/PBS/NaN_3 (<0.1%w/v)).
BUF WASH x20	1 x 100ml	Wash Buffer Concentrate (20X) for 2L, (clear solution, PBST)
DIL SPE	1 x 50ml	Sample Diluent, ready to use (yellow solution, PBST/BSA/NaN_3 (<0.1%w/v))
CONJ IFAB	1 x 15ml	Conjugate, ready to use (clear solution, anti-Hu IgG-HRP/MOPS/Proclin)
SUB TMB	1 x 15ml	TMB Substrate, black vial, ready to use (clear/bluish solution, TMB/H_2O_2)
STOP ⚠	1 x 15ml	Stop Solution, white cap, ready to use (clear solution, 2.5% sulphuric acid)
	1	instruction leaflet
	1	Certificate of Analysis

5. Storage

The kit should be stored refrigerated at 2-8°C. Do not use the reagents beyond their expiry date. **Do not freeze.** Keep all reagents away from direct sunlight. Coated microwell strips are for one time use only. Unused microwell strips should be carefully resealed in the pouch containing desiccant to prevent condensation and stored at 2-8°C.

6. Sample Handling

AUTOZYME™ IFAB may be performed on human serum samples.
Preferably, use freshly collected serum samples. Do not use icteric, lipemic, haemolysed or bacterially contaminated samples. Sera with particles should be clarified by low speed centrifugation.
Blood samples should be collected in additive-free tubes. After separation from the clot, the serum samples should be used immediately, respectively stored at 2-8°C for two or three days, or frozen at -20°C for longer periods.

7. Additional Reagents and Equipment Required

Deionised or freshly distilled water.
Precision micropipettes to deliver 5 - 1000µl.
Multichannel micropipette or repeating dispenser to deliver 100µl.
2000ml measuring cylinders for reagent preparation.
Microplate reader (450nm reading filter + optional 620nm reference filter).
Automatic microplate washer capable of dispensing 300µl
Automation - The AUTOZYME™ IFAB ELISA may be processed with suitable automated ELISA analysers. Applications have to be validated prior to diagnostic use.

8. Procedural Precautions

Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Allow all reagents to equilibrate to room temperature (18 -25°C) before use.
Do not mix or substitute reagents or microwells from different lot numbers. This may lead to variations in the results. Always change tips between different calibrators, samples or control sera to prevent sample carryover. Never allow the same pipette tip to be used with different reagents. Special care is needed to prevent contamination of the substrate by the conjugate. Protect the substrate reagent from light to avoid increase in blank values. The substrate should be colourless to a pale blue hue. Any blue colouration (OD >0.050 at 620nm) indicates substrate contamination and the substrate should be discarded.
The well washing procedure is critical for the successful performance of the test, especially between conjugate and substrate incubations (i.e. the second and third incubations)
Do not use the kit beyond the expiry date given on the label.
Unused reagents are stable at 2-8°C for up to 56 days after first opening the container. However, multiple re-use could increase the risk of reagent contamination.

9. Assay Procedure

1. Prepare the **wash buffer** as follows: dilute contents of the wash buffer concentrate (x20) vial to 2000ml with deionised water.
2. Dilute patient samples 1/100 using the sample diluent e.g. 5µl sample added to 495µl diluent. The **kit calibrators and controls** do not require dilution.
3. Remove the antigen-coated microwells from the resealable sachet. Reseal any unrequired wells in the resealable sachet, along with the desiccant sachet.
4. For semi-quantitative determination, pipette 100µl of each **kit calibrator, control** and **diluted patient sample** into the appropriate wells. For semi-quantitative determination, pipette 100µl calibrator 1 (blank), calibrator 2 (Cut-off, yellow cap), controls and diluted patient samples in to the appropriate wells. Incubate for 30 minutes at room temperature (18°C to 25°C). It is recommended that calibrators and controls are tested in duplicate.
5. Discard the contents of the wells. Using the diluted wash buffer, wash the wells four (4) times with at least 300µl per well. Discard the contents of the wells and knock out the residue on absorbent material.
6. Pipette 100µl **conjugate** to each well and incubate for 30 minutes at room temperature.
7. Discard the contents of the wells. Using the diluted wash buffer, wash the wells four (4) times with at least 300µl per well. Discard the contents of the wells and knock out the residue on absorbent material.
8. Pipette 100µl **TMB substrate** into each well and incubate for 10 minutes. At room temperatures above 25°C the substrate incubation could be shortened, but should never fall short of 5 minutes.
9. Stop the reaction by adding 100µl of **stop solution**.
10. Measure the OD at 450nm. Bi-chromatic measurement with a reference wavelength at 620nm is recommended. Read OD values within 30 minutes of adding Stop Solution.

10. Calculation of Results

The concentrations of the patient samples can be determined by either of two methods:

Quantitative Determination

For each assay, prepare a calibration curve by plotting mean absorbance against calibrator concentration on linear graph paper, and interpolate unknowns. Alternatively, use a four parameter curve to plot the standard curve.

Semi-quantitative Determination

Run Calibrator 1, Calibrator 2 (Cut-Off), Kit Negative and Positive Controls and test samples.

(Test Sample OD - Cal 1 OD / Cal 2 OD - Cal 1 OD) x 12.5 = AU/ml.

The following serves only as a guide in the interpretation of the laboratory results.

Interpretation:

<9 AU/ml Negative

>9 AU/ml Positive

Any sample giving values above the calibrator range should be diluted and retested.

11. Quality Control

Good laboratory practice requires that quality control specimens be included in every run to check on assay performance. The kit control ranges are provided on the certificate of analysis. If either control value falls outside the quoted range, the results are invalid and the assay should be repeated.

Reagent blank (Calibrator 1) OD should be <0.05.

Calibrator 6 OD > 1.6.

Calibrator 2 (Cut-off) OD > Negative control OD < Positive control OD.

12. Performance

Precision

Samples measured twice per day for 20 days:

Conc	Repeatability		Between Day		Between Run		Within Device	
(AU/ml)	Std Dev	%CV	Std Dev	%CV	Std Dev	%CV	Std Dev	%CV
38.66	1.23	**3.2**	0.82	**2.1**	3.64	**9.4**	3.75	**9.7**
71.21	1.40	**2.0**	3.68	**5.2**	3.46	**4.9**	5.24	**7.4**
20.33	0.49	**2.4**	0.86	**4.2**	0.92	**4.5**	1.35	**6.6**

Repeatability precision for 4 levels (n = 20):

Sample 1: Mean = 3.275AU/ml, Std Dev = 0.2525AU/ml, %CV = 7.7%

Sample 2: Mean = 40.537AU/ml, Std Dev = 1.8553AU/ml, %CV = 4.6%

Sample 3: Mean = 18.882AU/ml, Std Dev = 0.5629AU/ml, %CV = 3.0%

Sample 4: Mean = 74.567AU/ml, Std Dev = 3.0407AU/ml, %CV = 4.1%

Clinical Sensitivity and Specificity

The utility of the Intrinsic Factor Antibody ELISA was evaluated by testing Pernicious anaemia patients alongside disease controls and 'normal' human sera. These results are summarised below.

Sensitivity = 100.0%

Specificity = 98.7%

Overall Agreement = 99.0%

PPV = 96.8%

NPV = 100.0%

LoD

The limit of detection (LoD) was determined based on 60 replicates of the blank and 10 replicates each of 6 low-level (NHS) samples. LoD was determined to be 0.1AU/ml.

Interference

Interference was studied by mixing sera with known Intrinsic Factor antibody levels for each isotype with potentially interfering serum samples and studying deviation from expected results. No significant interference was demonstrated for the following substances at the levels indicated: Haemoglobin (5g/L), Direct Bilirubin (300mg/L), Total Bilirubin (225mg/L), Rheumatoid Factor (100U/ml), Triglycerides (10g/L), Lipids and Ascorbate (µmol/L).

13. Safety Precautions

For *in vitro* diagnostic use only and Professional Use only.

Safety data sheets are available on request.

This product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following recommendations and precautions for maximum safety when handling.

The kit contains potentially hazardous components. Reagents may be irritating to eyes and skin thus avoid contact with eyes and skin. Do not smoke, eat or drink when manipulating the kit.

The **calibrators and controls** contain human source material. Although found negative when tested for HIV-1 and HIV-2 antibodies, HCV and hepatitis B surface antigen, no test can guarantee their absence. Therefore, the calibrators and controls should be handled using the same safety precautions employed when handling any potentially infectious material. In case of contact with any reagent, immediately flush eyes or skin with water. If ingested, wash out mouth with water and obtain medical attention immediately.

Used calibrators, controls, samples, pipette tips and plates should be handled as clinical waste and incinerated or disposed of in accordance with local rules. Other reagents should be diluted and flushed down the drain. It is recommended that gloves are worn when handling such items.

STOP

Stop Solution contains 2.5% v/v Sulphuric acid.

Signal Word: Warning

Hazard Statements: H314 - Causes severe skin burns and eye damage.

Precautionary Statements:

P280 - Wear protective gloves/ eye protection/face protection.

P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

14. References

Y Shoenfeld et al. (eds) *Diagnostic Criteria in Autoimmune Diseases*, ISBN: 978-1-60327-284-1, 2008 Humana Press, Totowa, NJ.

Test Procedure