



INTENDED USE

Anti-intrinsic factor is used for the semi-quantitative determination of IgG autoantibodies to intrinsic factor in human serum.

Intrinsic factor (IF), a glycoprotein containing sialic acid, plays an important role in the absorption of vitamin B12 (extrinsic factor) in the digestive tract. IF is produced by parietal cells located in the stomach mucosa. Following secretion into the stomach IF binds to vitamin B12 that was ingested with food. The vitamin B12-IF complex is absorbed in the ileum by binding to a specific receptor to IF. After absorption vitamin B12 is released into the blood stream where it binds to transcobalamin.

Reduced production of IF and/or impairment of its transport function bring about a deficiency in vitamin B12 that leads to the development of Biermer's anemia.

The chronic atrophic gastritis of type A (5% of all forms) is characterized by autoimmune processes that lead to the destruction of parietal cells and the production of autoantibodies to both parietal cells and IF. The gastritis of type A occurs frequently in context with other autoimmune polyendocrinic disorders (e.g. Hashimoto's thyroiditis, Addison's disease, insulin-dependent diabetes mellitus). Patients suffering from gastritis of type A face an elevated risk of carcinoma.

According to their binding sites autoantibodies to IF are divided into two types. Type 1 autoantibodies interact with the binding site for vitamin B12 and, therefore, interfere with the binding of vitamin B12 to the IF in the stomach. Otherwise, type 2 autoantibodies block the binding of both IF and vitamin B12-IF complexes to the specific receptor in the ileum by reacting with the corresponding site on the IF.

In contrast to RIA methods based on radioactive labeled vitamin B12 Anti-intrinsic factor Elisa determines both types of autoantibodies to IF and is not influenced by high concentrations of vitamin B12 in the sample.

Waters HM, Dawson DW, Horwarth JE, Geary CG: High incidence of type II autoantibodies in pernicious anaemia. J Clin Pathol (1993) 46 (1) : 45-7

Waters HM, Smith C, Horwarth JE, Dawson DW, Delamore IW. New enzyme immunoassay for detecting total, type I, and type II intrinsic factor antibodies. J Clin Pathol (1989) 42 (3) : 307-12.

PRINCIPLE of the TEST

Anti-intrinsic factor is an enzyme immunoassay for the semi quantitative determination of IgG autoantibodies to intrinsic factor.

The antibodies of the diluted patient samples and controls react with intrinsic factor immobilized on the solid phase of microtiter plates. Human recombinant intrinsic factor coated on the microtiter plate guarantees the specific binding of Anti-intrinsic factor IgG autoantibodies of the specimen under investigation. Following an incubation period of 60 min at room temperature (18...25°C), unbound serum components are removed by a washing step.

The bound antibodies react specifically with anti-human-IgG-antibodies conjugated to horse radish peroxidase (HRPO). Following an incubation period of 30 min at room temperature, excessive conjugate is separated from the solid-phase immune complexes by an additional washing step.

The horse radish peroxidase converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (H2SO4) into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound.

# Anti-intrinsic factor

- 96 determinations -



IVD In vitro diagnostic device

Enzyme immunoassay for the determination of IgG autoantibodies to intrinsic factor in human serum

<b>REF</b>	Catalogue number	<b>LOT</b>	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk



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## PATIENT SAMPLES

### Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation.

The samples may be kept at 2...8 °C for up to three days. Long-term storage requires -20°C.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20°C.

**Note:** *Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1000 µl sample diluent (C), prior to assay. (controls of the kit are ready for use, prediluted accordingly)*

## TEST COMPONENTS for 96 determinations

<b>A</b> <b>96</b>	<b>Microtiter plate</b> with 12 breakable strips per 8 wells (total 96) coated with intrinsic factor (human recombinant)	1 vacuum sealed with desiccant
<b>B</b> <b>BUF</b> <b>WASH</b>	<b>Concentrated wash buffer</b> , for 1000 ml solution <b>10x</b>	100 ml concentrate, capped white
<b>C</b> <b>DIL</b>	<b>Sample diluent</b>	100 ml ready to use, capped black
<b>D</b> <b>CONJ</b>	<b>Conjugate</b> contains anti-human-IgG (sheep), coupled to horse radish peroxidase	15 ml ready to use, capped red
<b>E</b> <b>SOLN</b> <b>TMB</b>	<b>Substrate</b> 3,3',5,5'-Tetramethylbenzidin in citrate buffer with hydrogen peroxide	15 ml ready to use, capped blue
<b>F</b> <b>H2SO4</b>	<b>Stop solution</b> 0.25 M sulfuric acid <b>0.25 M</b>	15 ml ready to use, capped yellow
<b>P</b> <b>CONTROL</b>	<b>Positive control</b> (diluted human serum)	1.0 ml ready to use, capped red
<b>CO</b> <b>CONTROL</b>	<b>Cut-off control</b> (diluted human serum)	1.0 ml ready to use, capped white
<b>N</b> <b>CONTROL</b>	<b>Negative control</b> (diluted human serum)	1.0 ml ready to use, capped green

### Materials required in addition

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- graduated cylinders
- distilled or de-ionized water

## Size and storage

Anti-intrinsic factor has been designed for 96 determinations.

The expiry date of each component is reported on its respective label, that of the complete kit on the box label.

Upon receipt, all components of the Anti-intrinsic factor have to be kept at 2...8°C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

## Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water.

The wash solution prepared is stable at 2...8°C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

All other assay components are ready for use and can be stored up to the expiry date stated on the label.

Avoid exposure of the TMB substrate solution to light!

## ASSAY PROCEDURE

- Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1000 µl sample diluent (C).
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (18...25°C) before use. Mix gently without causing foam.
2. Dispense  
**100 µl** controls (P, CO, N)  
**100 µl** diluted patient samples  
into the respective wells.
3. Seal plate, incubate **60 min** at room temperature.
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) to each well.
6. Seal plate, incubate **30 min** at room temperature.
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min in the dark** at room temperature.
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the optical density at **450 nm** versus 620 or 690 nm within **15 min** after adding the stop solution.

## DATA PROCESSING

Results are interpreted by calculating the binding index (BI) ratio:

$$BI = OD_{\text{sample}} / OD_{\text{cut-off control}}$$

This calculation can be done by the integrated evaluation software of the microplate reader used, too.

### Example of Typical Assay Results

Wells	OD (a)	OD (b)	OD (mean)	BI
Positive control	1.483	1.451	1.467	
Cut-off control	0.162	0.157	0.160	
Negative control	0.101	0.099	0.100	
Patient 1	1.218	1.186	1.202	7,5 – positive
Patient 2	0.108	0.116	0.112	0.7 – negative
Patient 3	0.171	0.161	0.166	1.0 – grey zone

### Test validity

The test run is valid if:

- the mean OD of the positive control is  $\geq 0.600$
- the mean OD of the negative control is  $\leq 0.200$
- the mean OD of the Cut-off control is  $\leq 0.500$

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

## REFERENCE VALUES

Anti-intrinsic factor	BI
positive	$> 1.0$
negative	$\leq 1.0$

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum levels, of Anti-intrinsic factor autoantibodies as usually done for other diagnostic parameters, too.

### Limitations of Method

Healthy individuals should be tested negative by the Anti-intrinsic factor. However, Anti-intrinsic factor autoantibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

## CHARACTERISTIC ASSAY DATA

### Calibration

There is no international reference material for autoantibodies to the intrinsic factor available. The kit is measured semi-quantitatively by calculation of a binding index ratio.

### Diagnostic Specificity and Sensitivity

Specificity and sensitivity data of the Anti-intrinsic factor have been determined by investigating 45 patients with no clinical signs of Biermer's anemia and 25 patients with the clinical diagnosis of Biermer's anemia.

Specificity: 91%  
Sensitivity: 98%

### Precision

#### Intraassay variability

8 determinations of each of 4 sera in one run:

Sample	mean OD	CV (%)
Serum 1	1.643	2.70
Serum 2	1.031	3.94
Serum 3	0.641	5.14
Serum 4	0.168	5.41

#### Interassay variability

3 determinations of each of 4 sera in 8 different runs:

Sample	mean OD	CV (%)
Serum 1	1.498	4.58
Serum 2	1.001	5.11
Serum 3	0.588	4.07
Serum 4	0.168	2.93

Remarks:

## INCUBATION SCHEME

# Anti-intrinsic factor (3600)

Dilute patient sera

10 µl serum + 1000 µl sample diluent (C)

1	<b>Bring all test reagents to room temperature (18...25°C)</b>					
2	Dispense	positive control (P) cut-off control (CO) negative control (N) 1 + 100 diluted patient sera	100 µl	100 µl	100 µl	100 µl
3	Incubate 60 min, room temperature (18...25°C)					
4	Wash Decant, 3 x 300 µl (made of B)					
5	Dispense conjugate (D)		100 µl	100 µl	100 µl	100 µl
6	Incubate 30 min, room temperature (18...25°C)					
7	Wash Decant, 3 x 300 µl (made of B)					
8	Dispense substrate (E)		100 µl	100 µl	100 µl	100 µl
9	Incubate in the dark 15 min, room temperature (18...25°C)					
10	Dispense stop solution (F)		100 µl	100 µl	100 µl	100 µl
11	Read at 450 against 620 (690) nm					

## SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2...8°C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1% w/v) and Kathon (1.0% v/v) as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum Anti-intrinsic factor levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned data only provide a guide to values which might be expected.