



INSTRUCTION MANUAL

REF 4087

January 29, 2008

GastrAK IgG Dot

- 20 x 3 determinations -



Immunodot for the determination of IgG antibodies to gliadin, tissue transglutaminase and mannan of *Saccharomyces cerevisiae* in human serum or plasma

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



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INTENDED USE

GastrAK IgG Dot is used for the qualitative determination of IgG antibodies to gliadin, tissue transglutaminase and mannan of *Saccharomyces cerevisiae* in human serum or plasma for the differential diagnosis of inflammatory bowel diseases.

The differential diagnosis of inflammatory bowel diseases to chronic diarrhea, recurrent abdominal dolor, infectious colitis, anorexia as well as the differentiation of CD to ulcerative colitis is still a high challenge.

Celiac disease, or gluten-sensitivity, is found already in neonates and is characterized by small intestinal damages leading to a so-called "flat" mucosa. Due to this extensive lesions mal-absorption occurs frequently accompanied with a depletion of key nutrients. Gliadin, the alcohol soluble fraction of gluten, represents the causative agent of celiac disease that provokes an inflammatory process in the small intestine. Gliadin is a substrate of tissue transglutaminase and cross-linked into high molecular complexes triggering probably both cellular and humoral immune responses. Incidence rates for celiac disease range from 1 in 300 (Western Ireland) to 1 in 4700 in European countries. However, a high number of subclinical cases of celiac disease have been detected by in-vitro tests revealing a prevalence of 4 in 1000. Individuals suffering from prolonged celiac disease additionally face an elevated risk of developing T cell lymphoma. In serum samples of celiac disease patients antibodies to gliadin and tissue transglutaminase are found. Especially IgA type antibodies are considered as specific markers of the disease. Since celiac disease is often accompanied by IgA deficiency, IgG type antibodies are the only serological parameters in diagnosis.

Non-specific inflammatory bowel diseases including Crohn's disease (Enteritis regionalis) are characterized by unknown etiology as well as chronic-remitting inflammatory processes of the intestine. The risk developing one of these diseases is strongly influenced by immunologic, genetic, infectious and environmental factors. Crohn's disease (CD) shows a wide spread inflammation of the gastro-intestinal tract with granuloma formation. The determination of IgA and IgG antibodies to *Saccharomyces cerevisiae* (baker's yeast) has been described as one important serological marker for the differential diagnosis of Crohn's disease recently. Up to 70 % of patients with CD show antibody levels to *Saccharomyces cerevisiae*. Although the cause for their occurrence has been unclear, antibodies to *Saccharomyces cerevisiae* (ASCA) are strongly associated with inflammatory processes of the intestine.

Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D: Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med*, 1997, 3, 797-801.
Conrad K, Schmechta H, Klafki A, Lobeck G, Uhlig HH, Gerdi S, Henker J: Serological differentiation of inflammatory bowel diseases. *Eur J Gastrol & Hepatol*.2002 14:129-135

PRINCIPLE of the TEST

GastrAK IgG Dot is a sensitive immunodot for the determination of IgG antibodies to gliadin, tissue transglutaminase and mannan of *Saccharomyces cerevisiae*.

Test strips are composed of nitrocellulose membrane fixed on a plastic support containing two control areas (positive and cut-off control) and 3 reaction areas coated with tissue transglutaminase (recombinant human), gliadin and mannan of *Saccharomyces cerevisiae* (purified).

Patient sera and strips are incubated in the test tray. During the first incubation antibodies of the patient sample bind to the target antigens immobilized on the solid-phase of the strips. Following an incubation period of 45 min unbound serum components are removed by a washing step.

The bound antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP). Following an incubation period of 45 min excessive conjugate is separated from the solid-phase immune complexes by an additional washing step.

HRP converts the colourless substrate solution into a dark purple precipitating product. After 10 minutes incubation while shaking the reaction is stopped by a washing step. The strips are dried for at least 30 min by pressing the reactive side onto absorbent paper. Results are regarded to be positive if the colouration of the test dot is more intense than the colouration of the cut-off control.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma samples can be used too. Lipaemic, hemolytic and contaminated samples should not be used.

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. The use of lipemic or hemolytic samples increases the background reaction and can lead to false positive results.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: *Neat patient samples have to be used and are diluted in the incubation tray (1 + 100).*

TEST COMPONENTS for 20 x 3 determinations

A	Dot strips	20 Dot strips
Ag	20 numbered strips coated with gliadin (purified), tissue transglutaminase (human recombinant) mannan (<i>Saccharomyces cerevisiae</i> , purified)	
B	Buffer, 5 fold	2 x 35 ml concentrate capped black
BUF	sufficient for 400 ml solution	
	5x	
D	Conjugate	40 ml ready to use capped red
CONJ	anti-human-IgG (sheep), conjugated with horse radish peroxidase	
	G	
E	Substrate	50 ml ready to use capped blue
SOLN	3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide (black bottle)	
TMB		
F	Incubation tray for 9 strips	2
G	Dot pattern	1

Materials required in addition

- micropipette 1000 - 5000 µl
- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- pipette tips
- graduated cylinders
- glassware
- distilled or de-ionized water
- horizontal plate shaker
- plastic pincers

Size and Storage

GastrAK IgG Dot has been designed for 20 x 3 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 GastrAK IgG Dot 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 and take dot strips with a plastic pincer.

Prepare a sufficient amount of buffer solution by diluting the concentrated buffer 5 times (1 + 4) with deionized or distilled water. For each dot strip 11 ml of buffer solution is required. For example, dilute 15 ml of the concentrate with 60 ml of distilled water per strip. The buffer solution prepared is stable at 2 - 8 °C up to 30 days.

Avoid exposure of the TMB substrate solution to light.

ASSAY PROCEDURE

Avoid any time shift during pipeting of reagents or neat samples.

1. Bring all reagents to room temperature (RT) (18-25°C) before use. Mix gently without causing foam. Note number of serum sample and strips as well as lot number and antibody isotype (IgG/ IgA).
2. Place the strips (A) with the reactive side up (labels on top). Dispense **1.5 ml** of buffer solution (made of B) into the respective wells.
3. Seal plate, incubate **5 min** on an horizontal shaker.
4. Add **15 µl neat** patient serum to the respective wells. Cover tray and incubate **45 min** while shaking at RT (18-25°C).
5. Decant or aspirate, wash each well **three times 5 min** with **1.5 ml** buffer solution (made of B) while shaking. (Discard the solution contained in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper.)
6. Add **1.5 ml** of the conjugate (D) to each well.
7. Cover tray, incubate **45 min** at RT (18-25°C) while shaking.
8. Decant or aspirate, wash each well **three times 5 min** with **1.5 ml** buffer solution (made of B) while shaking. (Discard the solution contained in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper.)
9. Add **1.5 ml** of substrate (E) to each well.
10. Cover tray, incubate **10 min** on a horizontal shaker at RT (18-25°C) till a blue color develops.*
11. Decant or aspirate, wash the strips **three times** with **1.5 ml** distilled water to stop the reaction.*
12. Collect the strips from the wells and dry the membrane by pressing briefly the reactive side of the strip on the absorbent paper. After approximately 30 min the strips are interpreted by means of the lot specific pattern and the cut-off line.

* **Some sera may show a quick unspecific background coloration of the strip. In this case the reaction has to be stopped quickly by rinsing the strips three times with 1.5 ml distilled water.**

DATA PROCESSING

Evaluation criteria

Results should be interpreted only if the specific control line is clearly and the cut-off line weakly visible.



Evaluation

Only dried dot strips should be interpreted. Interpretation should be done within 6 hours after stopping the substrate reaction because weak colored lines may disappear.

The interpretation of the dot strips is based upon the comparison of the developed antigen lines and the cut-off line according to the table below.

	GastrAK IgG Dot
negative	antigen coloration < cut-off line
positive	antigen coloration ≥ cut-off line

Limits of the method

Healthy individuals should be tested negative by the GastrAK IgG Dot. However, 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.

In all immunologic methods bacteriological or fungal contaminations of the kit components and samples as well as cross-reactivity may cause false results.

Insufficient washing or time management may lead to incorrect results.

CHARACTERISTIC ASSAY DATA

Clinically defined sera were detected in GastrAK IgG Dot. Results were compared with results obtained using another commercial line dot assay.

Gliadin IgG

		Comparison assay	
		positive	negative
GastrAK IgG	positive	8	4
	negative	2	22

Tissue transglutaminase IgG

		Comparison assay	
		positive	negative
GastrAK IgG	positive	5	8
	negative	1	22

Mannan (ASCA) IgG

		Comparison assay	
		positive	negative
GastrAK IgG	positive	4	2
	negative	1	29

Remarks:

INCUBATION SCHEME

GastrAK IgG Dot (4087)

Dot strips, buffer solution and substrate can be used for the **GastrAK IgA Dot (4086)**

1.	Bring all reagents and the requested number of strips to RT (18-25°C)
2.	Place the strips with the reactive side upside in the tray and dispense 1.5 ml of buffer solution (made of B) into the respective wells
3.	Cover tray and incubate while shaking 5 min, RT (18-25°C)
4.	Pipette 15 µl neat patient serum
5.	Cover tray and incubate while shaking 45 min, RT (18-25°C)
6.	Decant, wash strips while shaking 3 x 5 min with 1.5 ml (made of B)
7.	Pipette 1.5 ml conjugate (D)
8.	Cover tray and incubate while shaking 45 min, RT (18-25°C)
9.	Decant, wash strips while shaking 3 x 5 min with 1.5 ml (made of B)
10.	Pipette 1.5 ml substrate (E)
11.	Incubate while shaking 10 min, RT (18-25°C)
12.	Decant, wash strips 3 x with 1.5 ml Aqua dest.

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 kit should be performed by trained technical staff only.
- 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 preservatives. 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 for HIV as well as 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.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.