

EIAgen Anti-Gliadin IgA Kit

[REF] 11AGA-A

S 96 Tests

M Adaltis Italia S.p.A.


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FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2...8 °C

EN

SYMBOLS USED ON LABELS

[_MT_PLATE_]	Microplate
[_CONJ_][_HRP_]	Conjugate
[_CAL_0_]	Calibrator 0
[_CAL_1_]	Calibrator 1
[_CAL_2_]	Calibrator 2
[_CAL_3_]	Calibrator 3
[_CAL_4_]	Calibrator 4
[_CAL_5_]	Calibrator 5
[_DILSPE_][_15x_]	Sample Diluent 15x
[_WASH_][_BUF_][_15x_]	Washing Buffer 15x
[_SOLN_][_TMB_]	Substrate (TMB)
[_SOLN_][_STOP_]	Stop Solution
[LOT]	Lot number
[REF]	Catalogue Code
[_CONTROL_][_+_]]	Positive Control
[_CONTROL_][_ - _]	Negative Control
[_CUT__OFF_]]	Cut Off
e	Expiry date (Use by...)
t ^{8°C} 2°C	Temperature limitation (Store at 2...8°C)
S	Number of tests
	Keep away from sunlight
M	Manufactured by
!	Attention, See Instruction For Use
D	Biological risks
[_IVD_]]	In vitro diagnostic medical device (In vitro diagnostic use)

1.0 INTENDED USE

The EIAgen Anti-Gliadin IgA kit is a rapid ELISA method for the detection of antibodies to gliadin, one of the principal components of wheat, barley and rye gluten, in human serum or plasma. The components of the kit are for "in vitro" diagnostic use only.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Gliadin is a mixture of glutamine containing, alcohol soluble proteins, termed prolamins, present in wheat, barley and rye gluten. These proteins are associated with the harmful effects of celiac disease and gluten-sensitive enteropathy in humans. The proteins cause characteristic changes in the small intestinal mucosa. If patients are placed on a strictly gluten-free diet, the symptoms of the disease can be avoided. Serum IgG and IgA antibodies to gliadin have been closely associated with celiac disease

3.0 PRINCIPLE OF THE ASSAY

Diluted serum samples are incubated with gliadin immobilised on microtitre wells. After washing away unbound serum components, rabbit anti-human IgA conjugated to horseradish peroxidase (HRP) is added to the wells, and this binds to surface-bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of Stop Solution terminates the reaction and provides the appropriate pH for colour development. The optical densities (OD) of the calibrators, controls and samples are measured using a microplate reader at 450nm. Optical density is directly proportional to antibody activity in the sample.

4.0 KIT CONTENTS

All reagents should be refrigerated at 2...8°C

4.1 MICROPLATE (code 36PIA) [_MT_PLATE_]

1 Microplate of 12 strips of 8 breakable wells, pre-coated with gliadin, with holder in a foil bag with desiccant

4.2 CONJUGATE (code 36HRP) [_CONJ_][_HRP_]

1 vial containing 12 mL of rabbit anti-human IgA (yellow) conjugated to HRP in protein stabilising solution and antimicrobial agent.
Ready to use.

4.3 CALIBRATORS (code 36CAL0-5)

6 vials containing 1,5 mL of human serum IgA antibodies to Gliadin in 10mM Tris-buffered saline.
Ready to use.

The concentrations of the calibrators are as follows:

Calibrator	symbol	code	concentration
Calibrator 0	[_CAL_0_]	36CAL0	0 U/mL
Calibrator 1	[_CAL_1_]	36CAL1	6.25 U/mL
Calibrator 2	[_CAL_2_]	36CAL2	12.5 U/mL
Calibrator 3	[_CAL_3_]	36CAL3	25 U/mL
Calibrator 4	[_CAL_4_]	36CAL4	50 U/mL
Calibrator 5	[_CAL_5_]	36CAL5	100 U/mL

4.4 POSITIVE CONTROL (code 36POS) [_CONTROL_][_+_]]

1 vial containing 1.5 mL of 10mM Tris-buffered saline containing human serum antibodies to gliadin.
Ready to use.

4.5 NEGATIVE CONTROL (code 36NEG) [_CONTROL_][_ - _]

1 vial containing 1.5 mL of 10mM Tris-buffered saline containing normal human serum.
Ready to use.

4.6 CUT OFF (code 36CO) [_CUT__OFF_]]

Cut-off for qualitative use: 1 vial containing 1,5 mL of human serum IgA antibodies to gliadin (4 U/mL) in 10mM Tris-buffered saline containing.
Ready to use.

4.7 SAMPLE DILUENT (code 36SD) [_DILSPE_][_15x_]

1 vial containing 15 mL of concentrated (15x) sample diluent (150mM TRIS-buffered saline, pH7.2, with antimicrobial agent).
Prepare the working Sample Diluent by diluting it to 225 mL with distilled water(1:15).

4.8 WASHING BUFFER (code TLAVG) [_WASH_] [_BUF_] [15x]

1 bottle containing 75 mL of concentrated washing solution (150mM TRIS-buffered saline with detergent, pH7.2).

Dilute the Wash Buffer 1 to 15 in distilled water to make sufficient buffer for the assay run.

STORAGE: The diluted wash buffer is stable for three months at 2...8 °C. Concentrated wash buffer is stable until the expiration date of the kit at 2...8°C.

4.9 SUBSTRATE (TMB) (code TMBG) [_SOLN_] [_TMB_]

1 vial containing 12 mL of aqueous solution of TMB and hydrogen peroxide.

Ready to use.

4.10 STOP SOLUTION (code STOPG) [_SOLN_] [_STOP_]

1 vial containing 12 mL of 0.25M sulphuric acid.

Ready to use.

The MSDS is available upon request of laboratory personnel.

5.0 STORAGE AND STABILITY AFTER THE FIRST OPENING

On arrival, store the kit at 2 - 8°C. Do not use kits beyond their expiry date.

Do not freeze any kit component.

After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2...8°C.

Once opened the kit is stable for 3 months (or until its expiry date if less than 3 months).

6.0 MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 10µL, 100µL, 1mL • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable automated system may be used.

Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

7.0 WARNINGS AND PRECAUTIONS

7.1 SAFETY PRECAUTIONS

1. All reagents in this kit are for *in vitro* diagnostic use only.
2. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
3. All human source material used in the preparation of calibrators and controls for this product have been tested and found negative for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
4. Reagents of this kit contain antimicrobial agents and the TMB Substrate contains 3,3', 5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
5. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
6. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local legislation.
7. Do not pipette by mouth.
8. Do not smoke, eat or apply cosmetics in areas in which specimens or kit reagents are handled.
9. Avoid microbial contamination of reagents during pipetting by using disposable pipette tips.
10. Please refer to the MSDS for further information about dangerous components.

7.2 TECHNICAL PRECAUTIONS

A. Correct use of reagents and proper pipetting

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 – 8°C after use.
3. Include the Positive and Negative Control in every test run to monitor for reagent stability and correct assay performance.
4. Strictly observe the indicated incubation times and temperature.
5. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette.
6. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate.
7. When pipetting Enzyme Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
8. Do not allow microwells to dry between incubation steps.
9. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.

10. Avoid direct sunlight and exposure to heat sources during all incubation steps.
11. Replace colour-coded caps on their correct vials to avoid cross-contamination
12. It is important to dispense all samples and controls into the wells without delay. Therefore, ensure that all samples are ready to dispense.
13. The sample diluent 15x concentrate contains 0.09% sodium azide as preservative. Prepare sufficient working strength diluent for the assay run. However, if the working strength diluent is to be stored for more than 1 week, add sodium azide (0.9g/L). Store unused sample diluent concentrate and dilute sample diluent at 2 – 8°C.

B. Adherence to assay procedure and specifications

1. The obtained values have to be always compared to the ones reported in QC sheet.
2. Do not use the kit to determine values outside the range indicated in the IFU.
3. The test protocol must be followed strictly. Observe the indicated incubation times and temperatures and the washing procedure, these are critical steps.
4. Include the controls in every test run to monitor for reagent stability and correct assay performance.

8.0 SPECIMEN COLLECTION AND STORAGE

Serum or plasma samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

9.0 ASSAY PROCEDURE

9.1 REAGENT PREPARATION

- Ensure that all materials are at room temperature before beginning the procedure.
- Assemble the number of strips required for the assay. It is recommended that the calibrators and blanks be run in duplicate. Samples may be run singly or in duplicate.
- Dilute the Sample Diluent 1:15 in distilled water to make sufficient buffer for the assay run.
- Dilute the Wash Buffer 1:15 in distilled water to make sufficient buffer for the assay run.
- Dilute patient samples 1:101 (e.g. 10µL serum plus 1 mL diluent) in sample diluent before assay.
- It is important to dispense all samples and calibrators into the wells without any delays. Ensure that all samples are ready to dispense. Slow pipetting or breaks in the flow of pipetting can cause drift. If it is necessary to stop the flow of dispensing, for any reason, a second set of calibrators should be dispensed.

9.2 PIPETTING AND INCUBATION STEPS

- A. Assemble the number of strips for the assay.
- B. For quantitative assays, dispense 100 µl of each calibrator, the Negative and Positive Controls and the diluted patient samples into appropriate wells.
- C. For qualitative assays, dispense only the cut-off, together with controls and samples
- D. **Incubate** the strips for **30 minutes** at room temperature.
- E. After 30 minutes, **decant** or **aspirate** the well contents and wash the wells 3 times using automated washing or the manual wash procedure (see below).
Careful washing is the key to good results. Do not allow the wells to dry out.
Manual Wash Procedure:
Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with Wash Buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times.
- F. **Dispense** 100µl of Conjugate into each well.
- G. **Incubate** the wells for **30 minutes** at room temperature.
- H. After 30 minutes, **discard** the well contents and carefully wash the wells with Wash Buffer. **Repeat** the washing stage 3 more times. Ensure that the wells are completely washed. **Dry** the microplate on absorbent paper to remove final drops of wash fluid.
- I. **Dispense** 100µl of Substrate into each well.
- J. Incubate the plate for **10 minutes**
- K. **Add** 100µl of Stop Solution to each well. In order to allow each well the same development time, the Stop Solution should be added to the wells in the same order sequence as the Substrate.
- L. **Read** the optical density at 450 nm in a microplate photometer within 10 minutes. A 620 nm filter may be used as a reference wavelength.

10.0 CALCULATION OF RESULTS

10.1 VALIDITY OF THE ASSAY

In order that the assay be valid, the following criteria must be fulfilled:

- Adaltis supplies with each EIAgen Anti-Gliadin IgA kit, positive and negative control samples which should be assayed with each run. The results of these quality control samples should fall within the limits indicated on the Certificate of Analysis.
- Results obtained for quality control sera must fall within acceptable ranges: please refer also to next section

10.2 QUALITY CONTROL

Quality control data is supplied on the lot - specific QC certificate included in the kit. Controls are intended to monitor for substantial reagent failure.

Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

Good laboratory practice indicates that with each assay run, one or more quality control samples of known antibody level should be analysed as though they were clinical samples.

Should the results fall outwith this range the assay should be repeated using freshly prepared controls. Should the results continue to fall outside the specific range, and after equipment, adherence to the protocol and laboratory procedure have been verified, assistance from the supplier should be sought. Do not report patient results if the control results fall outwith the acceptable ranges.

10.3 QUALITATIVE RESULTS

Samples with OD higher than the one of the Cut off (4 U/mL), are positive.

10.4 QUANTITATIVE CALCULATION

Plot the OD of each calibrator against its concentration and draw the best-fit curve through the points. Read the unknowns off this curve.

Alternatively, use a 4-parameter logistic curve fitting for the calibrator curve and for calculating results.

Each laboratory should establish its own normal range data. Values above 100 U/mL should be re-assayed at a higher dilution.

11.0 EXPECTED VALUES

Data based on 159 adult donors and 86 children.

	Gliadin IgA
Adults >16 years (n=159)	0 – 4 U/mL
Children 0-16 years (n=86)	0 – 4 U/mL

Note: For gliadin IgA, samples with gliadin IgA values between 3-4 U/mL are indeterminate.

- For gliadin IgA determinations, it is important to know the IgA status of a patient by measuring the total serum IgA level, as there is a high incidence of IgA deficiency in celiac disease.
- Anti-gliadin IgA or tissue transglutaminase IgA should be evaluated in all patients with gliadin IgG values above 10 U/mL (30 U/mL for children) as a number of patients exhibit raised gliadin IgG in the absence of celiac disease.
- Results should be interpreted with caution in patients with Down's syndrome and systemic autoimmune disease, as these groups are also associated with raised anti-gliadin antibodies.

12.0 LIMITATIONS OF THE PROCEDURE

12.1 KNOWN INTERFERENCES

Grossly haemolysed, icteric or lipaemic specimens should be avoided.

12.2 CAUTIONS IN INTERPRETATION OF THE RESULTS

Results must be interpreted in conjunction with other clinical information relating to the patient.

A negative result should not be used as a sole criterion to rule out autoimmune disease, but must be taken in relation to other clinical observations and diagnostic tests. While the precision of the EIAgen kit is sufficient to allow samples to be measured in single determinations, this is done at the clinical laboratory's discretion. It is advised that duplicate determinations should be used to enable identification of potential pipetting error or to allow for confirmation in the equivocal range.

13.0 PERFORMANCE CHARACTERISTICS

13.1 PRECISION

Within assay precision

	Mean	CV%	n
IgA Positive	134.5	4	10
IgA Negative	3.2	3	10

Between assay precision

	Mean	CV%	n
IgA Positive	140	7	3
IgA Negative	3.1	8	3

13.2 ANALYTICAL SPECIFICITY AND SENSITIVITY

Based on analysis of 53 confirmed disease positive celiac patients, 35 patients with treated celiac disease and Dermatitis Herpetiformis, 90 non-celiac controls and 194 adult blood donors.

Gliadin IgA	Disease Positive	Disease Negative
Positive	34	13
Negative	19	306

Sensitivity: 64.2%

Specificity: 95.9%

14.0 AUTOMATION

Application protocols for the proper automation on the Adaltis microplate analyzers are available upon request at Adaltis directly.

15.0 SUGGESTIONS FOR TROUBLESHOOTING

Adherence to assay procedure and specifications, as well as a correct use of reagents and proper pipetting, may help to avoid the following kinds of errors .

ERROR	POSSIBLE CAUSES / SUGGESTIONS
OD very different (\pm 50%) from OD reported on QC	<ul style="list-style-type: none"> - incorrect dispensing volume of reagents (suggestion: check the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes) - incorrect temperature or incorrect incubation time (suggestion: more care in the incubator maintenance; note down the beginning of the incubation) - error in washing or in spectrophotometer reading (suggestion: check operating or settings of respective instruments) - contamination of Substrate (suggestion: use only disposable and clean plastic containers)
Low reproducible results	<ul style="list-style-type: none"> - not constant dispensing volume of samples or reagents (suggestion: check the pipettes precision and the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes) - error in washing or in reading (suggestion: check operating or settings of respective instruments) - contamination of Substrate (suggestion: use only disposable and clean plastic containers) - pollution or degradation of reagents (suggestion: use appropriate tips, disposable and clean plastic containers for reagents and high quality distilled or equivalent water)
no colorimetric reaction after addition of substrate	<ul style="list-style-type: none"> - no reagent pipetted - strong contamination of conjugate or Substrate - errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)
too low reaction (too low ODs)	<ul style="list-style-type: none"> - incorrect conjugate (e.g. not from original kit) - incubation time too short, incubation temperature too low
too high reaction (too high ODs)	<ul style="list-style-type: none"> - incorrect conjugate (e.g. not from original kit) - accidental contamination/degradation of conjugate - incubation time too long, incubation temperature too high - water quality for wash buffer insufficient (low grade of deionization) - insufficient washing (conjugates not properly removed)
unexplainable outliers	<ul style="list-style-type: none"> - contamination of pipettes, tips or containers - insufficient washing (conjugates not properly removed)
too high within-run CV%	<ul style="list-style-type: none"> - reagents and/or strips not pre-warmed to Room Temp. prior to use - plate washer is not washing correctly (suggestion: clean washer head)
too high between-run CV%	<ul style="list-style-type: none"> - incubation conditions not constant (time, temperature) - controls and samples not dispensed at the same time (with the same intervals) - (check pipetting order) - person-related variation

16.0 BIBLIOGRAPHY

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