ElAgen Anti-Gliadin IgG Kit

**IFU 11AGA-G_rev.01 (07/2004)**

**1.0 INTENDED USE**

The ElAgen Anti-Gliadin IgG kit is a rapid ELISA method for the detection in human serum or plasma of antibodies to gliadin, one of the principal components of wheat, barley and rye gluten. 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 IgG 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 proportional to antibody activity in the sample.

**4.0 KIT CONTENTS**

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

**4.1 MICROPLATE (code 37PIA)**

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

**4.2 CONJUGATE (code 37HRP)**

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

**4.3 CALIBRATORS (code 37CAL0-5)**

6 vials containing 1.5 mL of different concentrations of human serum IgG antibodies to Gliadin in 10mM Tris-buffered saline. Ready to use.

The concentrations of the calibrators are as follows:

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>symbol</th>
<th>code</th>
<th>concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator 0</td>
<td>[CAL_0]</td>
<td>37CAL0</td>
<td>0 U/mL</td>
</tr>
<tr>
<td>Calibrator 1</td>
<td>[CAL_1]</td>
<td>37CAL1</td>
<td>6.25 U/mL</td>
</tr>
<tr>
<td>Calibrator 2</td>
<td>[CAL_2]</td>
<td>37CAL2</td>
<td>12.5 U/mL</td>
</tr>
<tr>
<td>Calibrator 3</td>
<td>[CAL_3]</td>
<td>37CAL3</td>
<td>25 U/mL</td>
</tr>
<tr>
<td>Calibrator 4</td>
<td>[CAL_4]</td>
<td>37CAL4</td>
<td>50 U/mL</td>
</tr>
<tr>
<td>Calibrator 5</td>
<td>[CAL_5]</td>
<td>37CAL5</td>
<td>100 U/mL</td>
</tr>
</tbody>
</table>

**4.4 POSITIVE CONTROL (code 37POS)**

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 37NEG)**

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

**4.6 CUT OFF (code 37CO)**

Cut-off for qualitative use: 1 vial containing 1.5 mL of human serum antibodies to gliadin (10 U/mL) in 10mM Tris-buffered saline. Ready to use.

**4.7 SAMPLE DILUENT (code 37SD)**

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)**

1 bottle containing 75 mL of concentrated (15x) washing solution (150mM TRIS-buffered saline with detergent, pH7.2).

Add 225 mL of distilled water to the Washing Buffer to make sufficient buffer for the assay run.
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 temperatures and the washing procedure, these are critical steps.

9. Avoid microbial contamination of reagents during pipetting by using disposable pipette tips.

10. Please refer to the MSDS for further information about dangerous reagents are handled.

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 X15 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. Microbiologically contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipemic 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 assay, 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.

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.

E. Dispense 100µL of Conjugate into each well. The Eppendorf type of repeating dispensers are recommended.

F. Incubate the wells for 30 minutes at room temperature.

G. After 30 minutes, discard the well contents and carefully wash the wells with Wash Buffer. Repeat the washing stage three more times. Ensure that the wells are completely washed. Dry the microplate on absorbent paper to remove final drops of wash fluid.

H. Dispense 100µL of Substrate into each well.

I. Incubate the plate for 10 minutes.

J. 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.

K. 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.
13.0 PERFORMANCE CHARACTERISTICS

13.1 PRECISION

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>CV%</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG Positive</td>
<td>32.4</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>IgG Negative</td>
<td>9.3</td>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>

Between assay precision

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>CV%</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG Positive</td>
<td>141</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>IgG Negative</td>
<td>32.3</td>
<td>12</td>
<td>3</td>
</tr>
</tbody>
</table>

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-ceeliac controls and 194 adult blood donors.

<table>
<thead>
<tr>
<th>Gliadin IgG</th>
<th>Disease Positive</th>
<th>Disease Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>45</td>
<td>17</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>302</td>
</tr>
</tbody>
</table>

Sensitivity: 84.9% Specificity: 94.7%

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.

<table>
<thead>
<tr>
<th>ERROR</th>
<th>POSSIBLE CAUSES / SUGGESTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD very different (+ 50%) from OD reported on QC</td>
<td>- incorrect dispensing of reagents (suggestion: check the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes)</td>
</tr>
<tr>
<td>Low reproducible results</td>
<td>- 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)</td>
</tr>
<tr>
<td>No colorimetric reaction after addition of substrate</td>
<td>- no reagent pipetted</td>
</tr>
<tr>
<td>Too low reaction (too low ODs)</td>
<td>- strong contamination of conjugate or Substrate</td>
</tr>
<tr>
<td>Too high reaction (too high ODs)</td>
<td>- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)</td>
</tr>
<tr>
<td>Unexplainable outliers</td>
<td>- contamination ofSubstrate (suggestion: use only disposable and clean plastic containers)</td>
</tr>
<tr>
<td>Too high within-run CV%</td>
<td>- pollution or degradation of reagents (suggestion: use appropriate tips, disposable and clean plastic containers for reagents and high quality distilled or equivalent water):</td>
</tr>
<tr>
<td>Too high between-run CV%</td>
<td>- contamination ofSubstrate (suggestion: use only disposable and clean plastic containers)</td>
</tr>
</tbody>
</table>

16.0 BIBLIOGRAPHY

4. Greco, L et al, (1991) Multi-centre study on the frequency of identified cases of celiac disease in Europe and in the Mediterranean area. ESPGAN,
5. Capi, 11-12 October 1991