Version 22-11/24

User's Manual

Human Gliadin IgA ELISA Kit

REF **DEIA1749**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Gliadin IgA ELISA test system is intended for the qualitative and semi-quantitative detection of IgAclass antibodies to gliadin in human serum. The test system is intended to be used as an aid in the diagnosis of gastrointestinal disorders, mainly Coeliac Disease. This test is for research use only.

Principles of Testing

The Gliadin IgA Elisa test system is designed to detect IgA class antibodies to Gliadin in human sera. Wells of plastic microwell strips are sensitized by passive absorption with Gliadin antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgA is added to the wells and the plate is incubated. The Conjugate will react with Gliadin antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

Reagents And Materials Provided

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note: All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v).

1. **Plate.** 96 wells configured in twelve 1x8-well strips coated with Gliadin antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. **Conjugate.** Conjugated (horseradish peroxidase) goat anti-human IgA. Ready to use. One, 15 mL vial with a white cap.
3. **Positive Control (Human Serum).** One, 0.35 mL vial with a red cap.
4. **Calibrator (Human Serum).** One, 0.5 mL vial with a blue cap.
5. **Negative Control (Human Serum).** One, 0.35 mL vial with a green cap.
6. **Sample diluent.** One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphatebuffered-saline, (pH 7.2 ± 0.2). Green solution, ready to use. **Note:** Shake Well Before Use. Note: The Sample Diluent will change color in the presence of serum.
7. **TMB:** One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenadine (TMB). Ready to use. Contains DMSO < 15% (w).
8. **Stop solution:** One 15 mL bottle (red cap) containing 1M H_2SO_4, 0.7M HCl. Ready to use.

9. **Wash buffer concentrate (10X):** dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). Contains preservative **NOTE:** 1X solution will have a pH of 7.2 ± 0.2.

Materials Required But Not Supplied

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Pipettes capable of accurately delivering 10 to 200 µL
3. Multichannel pipette capable of accurately delivering (50-200 µL)
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant, (example: 10% household bleach, 0.5% sodium hypochlorite.)

Storage

1. Store the unopened kit at 2°C and 8°C.
2. **Coated microwell strips:** Store between 2°C and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.
3. **Conjugate:** Store between 2°C and 8°C. DO NOT FREEZE.
4. **Calibrator, Positive Control and Negative Control:** Store between 2°C and 8°C.
5. **TMB:** Store between 2°C and 8°C.
6. **Wash Buffer concentrate (10X):** Store between 2°C and 25°C. Diluted wash buffer (1X) is stable at room temperature (20°C to 25°C) for up to 7 days or for 30 days between 2°C and 8°C.
7. **Sample Diluent:** Store between 2°C and 8°C.
8. **Stop Solution:** Store at 2°C and 25°C.

Specimen Collection And Preparation

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or preservatives should be added. Avoid using hemolyzed,

lipemic, or bacterially contaminated sera.

4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2°C and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

Assay Procedure

1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2° and 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Neg. Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Pos. Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct blank well configuration.
6. Incubate the plate at room temperature (20-25 °C) for 25 ± 5 minutes.
7. Wash the microwell strips 5X.

A. Manual Wash Procedure:

a. Vigorously shake out the liquid from the wells.

b. Fill each well with wash buffer. Make sure no air bubbles are trapped in the wells.

c. Repeat steps **a.** and **b.** for a total of five washes.

d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (10% household bleach) at the end of the days run.

B. Automated Wash Procedure:

If using an automated microwell wash system, set the dispensing volume to 300-350 µL/well. Set the

wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100µL of the conjugate solution to each well at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as previously described in step 7.
11. Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

Quality Control

1. Each time the assay is run the Calibrator must be run in triplicate. A reagent blank, Negative Control, and Positive Control must also be included in each assay.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean. discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges:

	OD Range
Negative Control	≤ 0.250
Calibrator	≥ 0.300
Positive Control	≥ 0.500

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be < 0.9.

   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be>1.25.

   c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

Calculation

1. Correction Factor

A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component List located in the kit box.

2. Cut-off OD Value

To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.

(CF x mean OD of Calibrator = cutoff OD value)

3. Index Values or OD Ratios

Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2. Example:

Mean OD of Calibrator = 0.793

Correction Factor (CF) = 0.25

Cut off OD = 0.793 x 0.25 = 0.198

Unknown Specimen OD = 0.432

Specimen Index Value or OD Ratio = 0.432 / 0.198 = 2.18

Interpretation Of Results

Index Values or OD ratios are interpreted as follows:

	Index Value or OD Ratio
Negative Specimens	≤ 0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥ 1.10

Reference Values

To establish or estimate the expected reactivity rate, the 305 specimens, which were tested in-house, were analyzed. This represented two groups of specimens; 255 clinical specimens which were either sent to the lab for routine gliadin serological analysis or were part of an external gliadin study, and 50 random normal donor specimens.

With respect to the clinical population, 107/255 (42.0%) were positive, 140/255 (54.9%) were negative, and 8/255 (3.1%) were equivocal.

With respect to the normal population, 49/50 (98.0%) were negative. 1/50 (2.0%) was positive.

Performance Characteristics

Comparative Study:

An in-house comparative study was performed to demonstrate the equivalence of the Gliadin IgA ELISA test system to another commercially available Gliadin IgA ELISA test system. Performance was evaluated using 305 specimens and the results are summarized in Table 1 below:

		CD Gliadin IgA ELISA			
		-	±**	+	Totals
Commercial ELISA Test System		175		7	184
	±**	8	2	9	19
	+	6	4	92	102
	Totals	189	8	108	305

Relative Sensitivity = 92/98 = 93.9%

95% Confidence Interval = 89% to 99%

Relative Specificity= 175/182 = 96.2%

95% Confidence Interval = 93% to 99%

Relative Agreement = 267/280 = 95.4%

95% Confidence Interval = 93% to 98%

** Data Excluded From Calculation

Precautions

1. For research use only.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered **potentially biohazardous materials** and handled accordingly.
4. The human serum controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen. HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodbome Pathogens.
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay.** Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.

7. The human serum controls, Sample Diluent, Conjugate, and Wash Buffer concentrate contain sodium azide at a concentration of 0.1% (w/v). Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing sodium azide.
8. The Stop Solution is **toxic**. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
9. The TMB Solution is **harmful**. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an **irritant**. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color. To help reduce the possibility of contamination, refer to Test Procedure, Substrate Incubation section to determine the amount of TMB to be used.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Wash solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: liquid waste at acid pH should be neutralized before adding to bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.