Human Gliadin IgA ELISA Kit

Cat.No:DEIA1749

Lot. No. (See product label)

PRODUCT INFOMATION

Storage	1. Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening, strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue. store between 2° and 8°C 2. Conjugate—DO NOT FREEZE. Store between 2° and 8°C 3. Unopened kit, calibrator, positive control, negative control, TMB, SAVe Diluent store between 2° and 8°C. 4. Stop Solution: Store between 2° and 25°C. 5. Wash Buffer (1X): 20° and 25°C for up to 7 days, 2-8°C for 30 days. 6. Wash Buffer (10X): 2° and 25°C
Pkg#Size	96T
Intended use	The Gliadin IgA ELISA test system is intended for the qualitative and semi-quantitative detection of IgA -class antibodies to gliadin in human serum. The test system is intended to be used as an aid in the diagnosis of gastrointestinal disorders, mainly Celiac Disease. This test is for in vitro diagnostic use.
Principle Of The Test	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): Controls, Calibrators and SAVe Diluent. 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. SAVe Diluent. One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-bufferedsaline, (pH 7.2 ± 0.2). Ready to use. Note: SAVe Diluent will change color when combined with serum. 7. TMB: One 15 mL amber bottle (amber cap) containing 3,3",5,5"-tetramethylbenadine (TMB). Ready to use. Contains DMSO 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). NOTE: 1X solution will have a pH of 7.2 ± 0.2. 1. NOTES: The following components are not kit lot number dependent and may be sued interchangeable with the Elisa kit system: TMB, Stop Solution, and Wash Buffer. 2. Kit also contains: a. Component Label containing lot specific information inside kit box. b. Package insert providing instructions for use.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

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/JL 3. Multichannel pipette capable of accurately delivering (50-200pL) 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.)
Assay Steps	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 returned to storage between 2° and 8 °C. 3. Prepare a 1:21 dilution (e.g.: 10 ul of serum ± 200 µL of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum. 4. To individual wells, add 100 uL 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 300-350µL/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 50ul 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 450 nm 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.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

Quality Control	1. Each time the assay is run, the low positive standard (LPS) must be run in triplicate. A high positive and negative control must also be included in each assay. 2. Calculate the mean of the three positive calibrator determinations. If any of the three positive calibrator values differ by more than 15% from the mean, discard that value and calculate the mean of the remaining two values. 3. The mean OD value for the positive calibrator and the OD values for the positive and negative controls should fall within the following ranges: OD Range Negative Control <> Positive Calibrator >0.300 Positive Control >0.500 a. The OD of the negative control divided by the mean OD of the positive calibrator should be <> b. The OD of the positive control divided by the mean OD of the positive calibrator should be >1.25. c. If the control values are not within the above ranges, the test should be considered invalid and the test 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.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

Precautions	1. 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. 2. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered POTENTIALLY BIOHAZARDOUS MATERIALS and handled accordingly. 3. 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 Institute of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (20). 4. 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. 5. 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. 6. The human serum controls, Sample Diluent, Conjugate, and Wash Buffer concentrate contain a preservative (sodium azide, 0.1% (w/v) react with laboratory plumbing which may cause explosion on hammering. 7. The Stop Solution is TOXIC. Causes burn. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately. 8. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin. 9. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin. 10. Wipe bottom of plate free of residual liquid and/or fingerprints, which can alter optical density (OD) readings. 11. Dilution or adulteration of these reagents may generate erroneous results. 12. Reagents from other sources or manufacturers should not be used. 13. 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. 14. Never pipette by mouth. Avoid contact or reagents and patient specimens with skin and mucous membranes. 15. Avoid microbial contamination of reagents. Incorrect results may occur. 16. Cross contamination of reagents and/or samples could cause erroneous results. 17. Reusable glassware must be washed out and thoroughly rinsed free of all detergents. 18. Avoid splashing or generation of aerosols. 19. Do not expose reagents to strong light during storage or incubation. 20. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation. 21. Wash solution should be collected in a disposal basin. Treat the waste solution with 10 household bleach (0.% sodium hypochlorite). Avoid exposure to reagents to bleach fumes. 22. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution. 23. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink. 24. Do not allow the conjugate to come in contact with containers or instruments, which may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity. 25. Do not expose any of the reactive reagents to bleach-containing solutions, or to any strong odors from bleachcontaining solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.
Specimen Collection And Handling	1. 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. 2. Only freshly drawn and properly stored blood 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. 3. 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° 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.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

Calculation	1. Correction Factor The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-today variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the kit box. 2. Cutoff 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 4. Conversion of Optical Density to AAU/mL: The conversion of OD to Unit Value (AAU/mL) can be represented by the following equation: Test Specimen AAU/mL = (A x B) / C where : AAU/mL = Unknown Unit Value to be determined; A = OD of the test specimen in question; B = Unit Value of the Positive Calibrator (AAU/mL) & C = the mean OD of the Calibrator. Example: Test Spcimen OD = 0.946 Test Specimen AAU/mL = (0.946 x 155) / 0.435 Calibraotr OD = 0.435 Test Sepcmen = 337 AAU/mL Calibrator Unit Value = 155 AAU/mL
Reference Values	Three hundred and five specimens were tested to establish or estimate the expected reactivity rate with the assay. 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.
General Description	Celiac disease is an inflammatory disorder of the small intestine induced by the prolamines of certain cereals, mainly the gliadins of wheat. This permanent intolerance to gliadin results in intestinal villous flattening and crypt hyperplasia in susceptible individuals. Immune reactions to gliadin are likely to play a role in the pathogenesis of the disease since both numeral and cell-mediated responses have been demonstrated in the peripheral blood and in the gut of coeliac patients. Classic signs of coeliac disease in adult include malabsorption characterized by weight loss, abdominal distension, diarrhea and steatorrhoea occurs because of the loss of absorptive area and the immaturity of surface epithelial cells. By the early 1980s, clinical features of coeliac disease have changed. There had been a shift towards milder symptoms such as indigestion in adults and recurrent abdominal pain in children. The classic symptoms and signs had become rare. And, despite manifest mucosal lesion, the disease can be even symptom-free, clinically silent. In children, it has become evident that the disease exists or appears late even though classical forms with malabsorption are not apparent .
Interpretation of Results	Index Values or OD ratios are interpreted as follows: Unit Values Index Value or OD Ratio Negative Specimens <> equivocal Specimens 150 to 180 AAU/mL 0.91 to 1.09 Positive Specimens >180 AAU/mL >1.10 Retest specimens with OD Ratio Values in the equivocal range (0.91 – 1.09) in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimens using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

Reproducibility	Technicians tested eight specimens in-house, to determine intra-assay and inter-assay variation: two strong positive specimens, two specimens near the cut-off zone, two low negative specimens and the kit's positive and negative controls. On each of three days, a technician tested each specimen once a day, eight times each, resulting in 24 test points. A responsible party then calculated the mean OD ration and coefficient of variation from the resulting data.
Limitations	1. A diagnosis should not be made solely on the basis of Gliadin ELISA results alone. The results for gliadin should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedure. 2. Crohn's disease and other food protein intolerance/gastrointestinal disorders may induce circulating antibodies to Gliadin and cause a false positive. 3. The clinical significance of any test result depends upon its relationship to other medical patient data. Disease diagnosis and management should be based on an evaluation of all relevant patient information. 4. Values for the pediatric population have not been established with this assay. 5. Gliadin IgA negative result in untreated patient does not rule out gluten-sensitive enteropathy when associated with high levels of gliadin IgG antibodies. The finding can often be explained by selective IgA deficiency, a relatively

Antigen Gene Information

Gene Name	LOC543191 alpha-type gliadin [Triticum aestivum]
Official Symbol	LOC543191
GeneID	543191

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com