ELISA kits available from ADI (see details at the web site)

Catalog#	ProdDescription
3100	Human anti-dsDNA IgG ELISA Kit, 96 tests, Quantitative
3105	Human anti-dsDNA IgM ELISA Kit, 96 tests, Quantitative
3110	Human anti-dsDNA IgA ELISA Kit, 96 tests, Quantitative
3115	Human anti-ssDNA IgG ELISA Kit, 96 tests, Quantitative
3205	Human Anti-Nuclear Antibodies (ANA) ELISA Kit, 96 tests, Semi-Quantitative
3210-SSA	Human anti-SS-A (60 Kda/Ro IgG ELISA Kit, 96 tests, Quantitative
3215-SSA	Human anti-SS-A (52 Kda/Ro IgG ELISA Kit, 96 tests, Quantitative
3220-SSB	Human anti-SS-B/La IgG ELISA Kit, 96 tests, Quantitative
3250	Anti-thyroid peroxidase ELISA kit, Semi-Quantitative
3300	Anti-helicobacter pylori IgG ELISA kit, Semi-Quantitative
3300-100-SMG	Human Anti-Smith antigen (Sm) IgG ELISA kit, 96 tests, Quantitative
3300-110-SRG	Human Anti-Smith antigen/RNP (Sm/RNP) IgG ELISA kit, 96 tests,
3300-120-RNG	Human Anti-RNP (RNP-70) IgG ELISA kit, 96 tests, Quantitative
3300-130-HNG	Human Anti-histones IgG ELISA kit, 96 tests, Quantitative
3300-140-SCG	Human Anti-Scl-70 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA kit,
3300-150-JOG	Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA kit, 96
3300-160-AFG	Human Anti-Alpha Fodrin IgG ELISA kit, 96 tests, Quantitative
3300-170-CLG	Human Anti-Cardiolipin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM	Human Anti-Cardiolipin IgM ELISA kit, 96 tests, Quantitative
3300-185-CLA	Human Anti-Cardiolipin IgA ELISA kit, 96 tests, Quantitative
3300-190-B2G	Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit, 96 tests, Quantitative
3300-195-B2M	Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit, 96 tests, Quantitative
3300-200-B2A	Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests, Quantitative
3300-205-APS	Human Anti-Phospholipid Screen (anti-Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and beta-2-Glycoprotein I) IgG/IgM ELISA kit, 96 tests, Quantitative
3300-210-PSS	Human Anti-Phosphotidyl serine IgG/IgM ELISA kit, 96 tests, Quantitative
3300-215-PIS	Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit, 96 tests, Quantitative
3300-220-PAS	Human Anti-Phosphotidic Acid IgG/IgM ELISA kit, 96 tests, Quantitative
3300-230-APG	Human Anti-Prothrombin IgG/IgM ELISA kit, 96 tests, Quantitative
3300-235-APA	Human Anti-Prothrombin IgA ELISA kit, 96 tests, Quantitative
3300-240-AVA	Human Anti-Annexin V IgG ELISA kit, 96 tests, Quantitative
3300-250-ANG	Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit, 96 tests,
3300-255-PRG	Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
3300-260-LFG	Human Anti-Lactoferrin IgG ELISA kit, 96 tests, Quantitative
3300-265-MPG	Human ANCA (Anti-MPO) IgG ELISA kit, 96 tests, Quantitative
3300-270-GBG	Human Anti-glomerular basement membrane (GBM) IgG ELISA kit, 96 tests,
3300-280-BPG	Human Anti-bactericidal permeability increasing (BPI) protein IgG ELISA kit, 96
3300-290-ELG	Human Anti-Elastase IgG ELISA kit, 96 tests, Quantitative
3300-300-GLG	Human Anti-Gliadin IgG ELISA kit, 96 tests, Quantitative
3300-305-GLM	Human Anti-Gliadin IgM ELISA kit, 96 tests, Quantitative
3300-310-GLA	Human Anti-Gliadin IgA ELISA kit, 96 tests, Quantitative
3300-315-PRG	Human Anti-Parietal cell (alpha and beta subunits of the Parietal Cell (H//K/ATPase) IgG ELISA kit, 96 tests, Quantitative
3300-320-ASC	Human Anti-ASCA (mannan from Saccharomyces cerevisiae) IgA/IgG ELISA kit, 96 tests, Quantitative
3300-330-ASG	Human Anti-Sperm IgG ELISA kit, 96 tests, Quantitative
3300-340-CCG	Human Anti-Cyclic Citrullinated Peptide (CCP) IgG ELISA kit, 96 tests,
3300-350-TPG	Human Anti-thyroid peroxidase (TPO) IgG ELISA kit, 96 tests, Quantitative
3300-360-TGG	Human Anti-thyroglobulin (TG) IgG ELISA kit, 96 tests, Quantitative

Instruction Manual No. M-3300-305-GLM

Human Anti-Gliadin IgM

ELISA KIT Cat. # 3300-305-GLM

For the determination of IgM class Autoantibodies against Gliadin in human serum or plasma.

For In Vitro Research Use Only

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Anti-Gliadin IgM ELISA KIT Cat. No. 3300-305-GLM

Kit Contents: (reagents for 96 tests)

Components	
Gliadin Antigen coated microwell strip plate (96 wells);#3300306	96 wells (1 plate)
Anti-Gliadin IgM **Std. A**, 1.5 ml, 0 U/ml, #3300307A	1 vial
Anti-Gliadin IgM **Std. B**, 1.5 ml, 6.3 U/ml, #3300307B	1 vial
Anti-Gliadin IgM **Std. C**, 1.5 ml, 12.5 U/ml, #3300307C	1 vial
Anti-Gliadin IgM **Std. D**, 1.5 ml, 25 U/ml, #3300307D	1 vial
Anti-Gliadin IgM **Std. E**, 1.5 ml, 50 U/ml, #3300307E	1 vial
Anti-Gliadin IgM **Std. F**, 1.5 ml, 100 U/ml, #3300307F	1 vial
Positive Control, 1.5 ml#3300305P (see lot sp. conc on the vial)	1 vial
Negative Control, 1.5 ml#3300305N (see lot sp. conc on the vial)	1 vial
Sample Buffer (5X) 20 ml, #3300308	1 bottle
Enzyme Conjugate, Anti-hIgM, 15 ml, #3300309	1 bottle
HRP **Substrate Solution**, 15 ml # #3300305TM	1 bottle
Wash buffer (50X), 20 ml, dilute 1:50 with distilled water #3300305-WB	1 bottle
Stop solution (ready-to-use), 15 ml, #3300305-ST	1 bottle
Complete Instruction Manual, M-3300-305-GLM	1

Intended Use

For the determination of IgM class Autoantibodies against Gliadin in human serum or plasma. For research use only (RUO), not for use in diagnosis, cure and prevention of the disease.

General information

Gliadin is a class of proteins present in wheat and several other cereals within the grass genus Triticum. Gliadins and gluten are essential for giving bread the ability to rise properly during baking. Gliadins and glutenins are the two main components of the gluten fraction of the wheat seed. There are three main types of gliadin (α, Y, and ω) and is what the body is intolerant to in coeliac (or celiac) disease, which is uncommon, but is becoming more diagnosed today. The α, Y, and ω gliadin types are separated and distinguished based on their amino acid sequences.α-/β-gliadins. Celiac disease is a genetic disease in which the body becomes intolerant to gluten, which is focused in the gliadin. Gliadin proteins have the ability to activate the disease in the person through the amino acid sequence found in the gliadin. The immune system responds negatively to the gliadin. Celiac disease starts as an intolerance to this protein and then expounds and extends. One of the problems with this disease is that it can go unrecognized for a long time, in which that time, it can cause severe damage to the body's digestive system and cause many more problems such as lactose intolerance. The main treatment for celiac disease is a gluten free diet in which the diseased person does not ingest any gluten, and specifically gliadin. There have been searches for an affordable and much better treatment, but the main treatment remains to abstain from ingesting any gluten.

Antigliadin antibodies are produced in response to Gliadin, a prolamin found in wheat. The IgG antibody is found at higher levels in patients with the IgA-less phenotype. It is also associated with celiac disease and idiopathic gluten sensitivity. Anti-gliadin antibodies are frequently found with anti-transglutaminase antibodies.

PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the Anti-Gliadin IgM kit. The assay shows linearity over the full measuring range.

Sensitivity

The lower detection limits for Anti-Gliadin IgM were determined at 0.5 U/ml.

Specificity

The microplate is coated with purified Gliadin from wheat. The test kit is specific only for antibodies against Gliadin.

Calibration

Since no international reference preparation for Anti-Gliadin autoantibodies is available, the assay system is calibrated in relative arbitrary units.

LIMITATIONS OF PROCEDURE

The Anti-Gliadin IgM ELISA is intended for research use only

INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolysed or lipemic samples should be avoided.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples MPL U/mL	Mean A_{450nm}
A1, A2	**Std. A** (0)	
B1, B2	**Std. B** (6.3)	
C1, C2	**Std. C** (12.5)	
D1, D2	**Std. D** (25)	
E1, E2	**Std. E** (50)	
F1, F2	**Std. F** (100)	

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.

A typical std. assay curve (do not use this for calculating sample values)

CALCULATION OF RESULTS:

For Anti-Gliadin IgM a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Recommended Lin-Log Plot: First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may Also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Quality Control

This test is only valid if the optical density at 450 nm for Positive Control and Negative Control as well as for the Standard A and F complies with the respective range indicated on the each vial If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

PRINCIPLE OF THE TEST

Purified gliadin from wheat is bound to microwells. Antibodies against this antigen, if Present in diluted serum or plasma, bind to the antigen.. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgM detect the bound antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyses to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of color is directly proportional to the concentration of IgM antibodies present in the original sample.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS:

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

REAGENTS PREPRATION:

Dilute wash buffer 20 ml with 980 ml distilled water**.** Store at 4oC.

Dilute Sample Buffer (5X) with 80 ml distilled water.

Sample preparation:

Dilute all samples **1:100** with **sample buffer** before assay. Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well. Controls are ready to use and need not be diluted.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually six months from the date of shipping, under appropriate storage conditions.

Sample preparation:

Dilute all samples **1:100** with **sample buffer** before assay. Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well. Controls are ready to use and need not be diluted.

QUALITY CONTROL

Each laboratory should utilize controls at several levels to monitor assay performance. The controls should be treated as unknown. Values obtained should be in a agreement with the assigned values of the control. Controls can be obtained from commercially available sources but should not contain sodium azide as preservative.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Dilute wash buffer & Sample Buffer as per detail on page 2 before use.**

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Label or mark the microtiter well strips to be used on the plate. Reference wells for identification.

2. Pipet **100 µl of standards**, control & pre-diluted patient samples, in appropriate wells in *duplicate*. Cover the plate and incubate for **30 minutes** at 20-28 oC.

3. Aspirate and wash the wells **3 times** with 300 µl wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Pipet **100 µl HRP-Conjugate** to each well. Cover the plate and incubate for **15 minutes** at 20-28 oC.

5. Aspirate and wash the wells **3 times** with 300 µl wash buffer as above.

6. Dispense **100 µl TMB substrate per well**. Mix gently for 5-10 seconds.

7. Cover the plate and incubate for **15 minutes** at 20-28 oC

8. Stop the reaction by adding 100 µl of stopping solution to all wells. Mix gently for 5-10 seconds. Blue color turns yellow. Read the plate at 450 nm within 30 min.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. do not touch the bottom of the wells.