Mouse Anti-Glutenin IgE Antibody ELISA Kit

Catalog # 3104

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse anti-glutenin IgE antibodies
FORMAT:	Precoated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4 hours
STANDARD RANGE:	50 – 0.8 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum & Plasma (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	1:10 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (0.4-2.2%)/Inter-Assay (4.7-7.1%)/Spiking Test (92-108%)
NOTES:	N/A

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INTRODUCTION

Wheat is the most widely consumed food grain in the world. Wheat proteins are categorized into four fractions based on their solubility in solvents: water (albumins), water containing salt (globulins), alcohol (gliadins), and alkali or acid solution (glutenins) (1).

One of these fractions, glutenins, are polymeric proteins composed of two main types of subunits: high-molecular-weight glutenin subunits (HMW-GS), with a molecular mass of 70–90 kDa, and low-molecular-weight glutenin subunits (LMW-GS), with a mass of 20–45 kDa. These subunits are interconnected by a vast network of intermolecular disulfide bonds, which are critical for forming the large, elastic glutenin polymers. HMW-GS and LMW-GS constitute approximately 30% and 60% of the polymer, respectively. Functionally, the HMW subunits are primarily responsible for the elasticity of the gluten complex, while the LMW subunits contribute to its viscosity. Furthermore, these subunits contain proline-rich regions that form unique secondary structures, making them resistant to complete digestion by human gastrointestinal enzymes(2).

Therefore, glutenin may play roles for triggering Celiac Disease (CD) which is an autoimmune disorder in genetically susceptible individuals. Many CD patients even have antibodies which recognize major epitopes (HQQQPIQQQP, QQPQQQPQQ, and QSRYEAIRAI) in LMW-glutenin(3). Mouse CD models have been widely used to study the pathogenesis of glutenin and its immune responses. The mice who receive glutenin have significantly higher serum glutenin specific IgE antibodies than controls (3-4).

To evaluate the humoral immunity against glutenin in mouse CD models, Chondrex, Inc. provides ELISA kits for assaying mouse anti-glutenin subtype and subclass antibodies, including IgE, IgM, IgG, IgG1, IgG2a, and IgG2b antibodies. Chondrex, Inc. also offers ELISA kits for assaying other allergens like anti-gliadin, ovalbumin and house dust mite antibody subtypes/subclasses as well as total immunoglobulin subtypes/subclasses. For more information, please visit www.chondrex.com or contact support@chondrex.com.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse Anti-Glutenin IgE Antibody (31041)	1 vial	50 ng, lyophilized	-20°C
Biotinylated Glutenin (31043)	1 vial	100 µl	-20°C
Solution C - Sample/Standard/Secondary Antibody Dilution Buffer (30106)	1 bottle	50 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	2 bottles	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
TMB Solution (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
Anti-Mouse IgE Antibody Coated ELISA Plate (Yellow)	1 each	96-well (8-well strips x 12)	-20°C

NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 100 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 50 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

NOTE 8: Serum IgE antibodies are a mixture of multiple antibodies with a variety of affinity ranges. The OD value obtained in ELISA for an antibody assay depends on the antibody concentration as well as the antibody affinity towards an antigen. In general, ELISA data using a monoclonal antibody as a standard does not reflect the absolute levels of IgE. Therefore, IgE levels determined using this kit should be expressed as pg of IgE per ml.

NOTE 9: If the total IgE concentration in a sample is higher than 500 ng/ml, the sample must be diluted to lower the total IgE levels below 500 ng/ml because the anti-glutenin IgE value obtained from this ELISA may be lower than the actual value due to competition from non-anti-glutenin IgE antibodies in sample. Therefore, it is strongly recommended that total IgE levels be determined first using the Mouse Total IgE Assay Kit (Cat # 3005).

PLATE MAPPING

	1	2	3	4	5	6	7	8	9	10	11	12
A	50	50	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	25	20	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	12.5	12.5	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	6.3	6.3	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	3.1	3.1	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	1.6	1.6	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	0.8	0.8	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Standards | Samples

ASSAY OUTLINE

Add 100 µl of diluted standards and samples

↓ Incubate at room temperature for 1.5 hours.
Wash plates.

Add 100 µl of diluted Biotinylated Glutenin

↓ Incubate at room temperature for 1.5 hours.
Wash plates.

Add 100 µl of diluted Streptavidin Peroxidase

↓ Incubate at room temperature for 30 minutes.
Wash plates.

Add 100 µl of TMB solution

↓ Incubate at room temperature for 25 minutes.

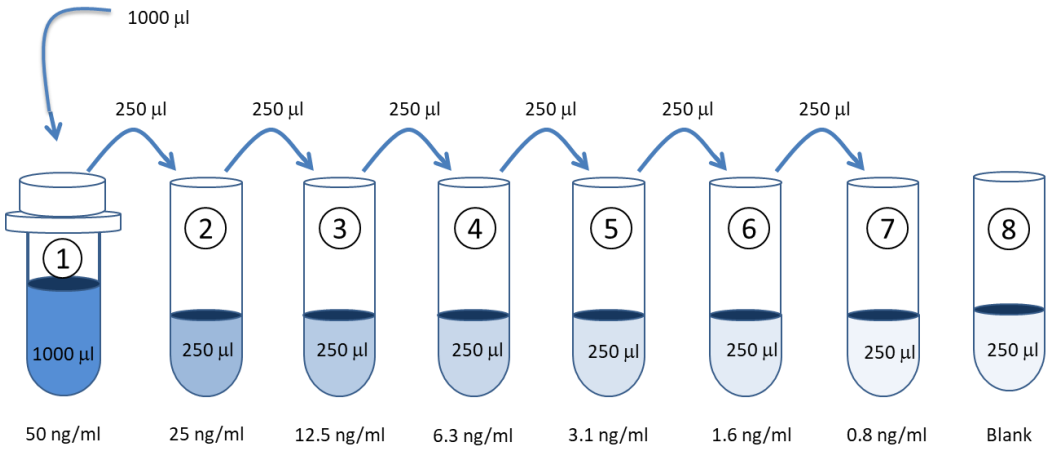
Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 0.8 - 50 ng/ml. Dissolve one vial of Standard (50 ng/vial) in 1 ml of Sample/Standard/Secondary Antibody Dilution Buffer (Solution C) and keep it as a standard stock. Then serially dilute it with Solution C. For example, mix 250 µl of the 50 ng/ml solution with an equal volume of Solution C to make a 25 ng/ml solution, and then repeat it five more times for 12.5, 6.3, 3.1, 1.6 and 0.8 ng/ml standard solutions.



2. **Prepare Sample Dilutions**: The serum dilution of mice immunized with glutenin will vary (1:10 or more) depending on the immunization schedule and timing of serum collection. In general, no IgE antibodies against glutenin are observed in normal serum at a 1:10 dilution.

3. **Add Standards and Samples**: Add 100 µl of standards, Solution C (blank), and samples to wells in duplicate. Incubate at room temperature for 1.5 hours.

4. **Wash**: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

5. **Add Biotinylated Glutenin**: Dilute one vial of biotinylated glutenin in 10 ml Streptavidin Peroxidase Dilution Buffer (Solution D). Add 100 µl of biotinylated glutenin solution to each well and incubate at room temperature for 1.5 hours.

Strip #	Biotinylated Glutenin (µl)	Solution D (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

6. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table. Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

8. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **Add TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

10. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

11. **Read Plate**: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

CALCULATING RESULTS

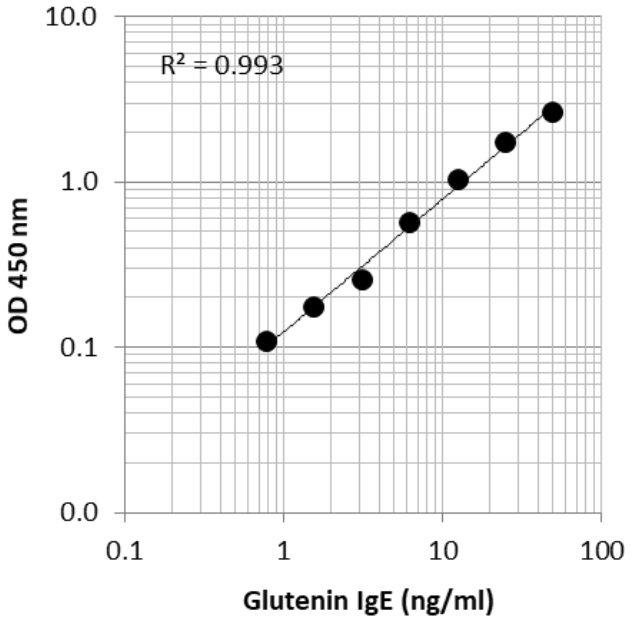
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

2. Subtract the averaged blank OD values from the averaged OD values of the standards and test samples.

3. Plot the OD values of the standards against the ng/ml of antibody standard. Using a log/log plot will linearize the data. Figure 1 shows an example of a standard curve for anti-glutenin IgE antibodies.

4. The ng/ml of antibody in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the antibody concentration (ng/ml) in the original test samples.

Figure 1 - A Typical Standard Curve for the Anti-Glutenin IgE Antibody ELISA Kit



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VALIDATION DATA

Table 1 - Reproducibility Data for the Mouse Anti-Glutenin IgE Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	2.2	0.4	1.8
Inter-Assay CV (%)	7.1	4.7	5.8
Spike Test* (%)	92%	108%	108%

*Known amounts of anti-glutenin IgE antibodies were added to samples and then diluted with Sample/Standard/Secondary Antibody Dilution Buffer to assay anti-glutenin IgE antibodies by ELISA.

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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