Mouse Anti-Gliadin Antibody Subtype/Subclass ELISA Kits

Catalog # 3051, 3052, 3053, 3054, and 3055

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse anti-gliadin antibodies
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	3.5 hours
STANDARD RANGE:	3051 (IgG) : 10 - 0.16 ng/ml 3052 (IgG1) : 50 - 0.8 ng/ml 3053 (IgG2a) : 10 - 0.16 ng/ml 3054 (IgG2b) : 10 - 0.16 ng/ml 3055 (IgM) : 50 - 0.8 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum & Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:1000 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	3051: Intra-Assay (2.2-7.7%)/Inter-Assay (5.5-8.6%)/Spiking Test (99-109%) 3052: Intra-Assay (3.0-8.4%)/Inter-Assay (6.8-8.7%)/Spiking Test (104-109%) 3053: Intra-Assay (3.5-4.8%)/Inter-Assay (1.8-4.5%)/Spiking Test (96-106%) 3054: Intra-Assay (1.8-4.1%)/Inter-Assay (4.0-8.3%)/Spiking Test (105-108%) 3055: Intra-Assay (2.5-3.4%)/Inter-Assay (2.3-4.9%)/Spiking Test (97-101%)
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 (glutelin) (1). One of these proteins, gliadin consists of alpha, beta, gamma, and omega types which are between 28 – 55kDa, and has repeated sequences which show high antigenicity. Gliadins play a critical role in activating both the innate and adaptive immune response, which results in the immune-mediated injury of the intestine such as high intestinal permeability and lamina propria inflammatory cell infiltration. Therefore, gliadins can trigger Celiac disease (CD) which is an autoimmune disorder in genetically susceptible individuals. Many CD patients even have antibodies which recognize major epitopes (QQFPQQQ, QQIPQQQ, and QQLPQQQ) in omega gliadin (2).

Mouse CD models have been widely used to study the pathogenesis of gliadin and its immune responses. The mice who receive gliadin have significantly higher serum gliadin specific IgE and IgG1 antibodies than controls (3–6). To evaluate the humoral immunity against gliadin in the mouse CD models, Chondrex, Inc. provides ELISA kits for assaying mouse anti-gliadin subtype and subclass antibodies, including IgE, IgM, IgG, IgG1, IgG2a, and IgG2b antibodies. Chondrex, Inc. also offers ELISA kits for assaying anti-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	IgG (30511) – 10 ng IgG1 (30521) – 50 ng IgG2a (30531) – 10 ng IgG2b (30541) – 10 ng IgM (30551) – 50 ng	1 vial	Lyophilized	-20°C
Secondary Antibody (peroxidase-conjugated polyclonal antibodies)	IgG (30113) IgG1 (30133) IgG2a (30153) IgG2b (30163) IgM (30173)	2 vials	50 µl	-20°C
Solution C - Sample/Standard/Secondary Antibody Dilution Buffer (30106)		2 bottles	50 ml	-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
Gliadin Coated ELISA Plate (Blue)		1 each	96-well (8-well strips x 12)	-20°C

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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 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 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: Depending on the isotypes, subtypes, and targeting epitopes of antibodies, the binding affinity of individual antibodies varies significantly. Therefore, the total IgG antibody concentration calculated as the sum of individual IgG subtypes might not perfectly match the total IgG concentration as determined by the Mouse Anti-Gliadin IgG Antibody ELISA Kit (Cat # 3051).

ASSAY OUTLINE

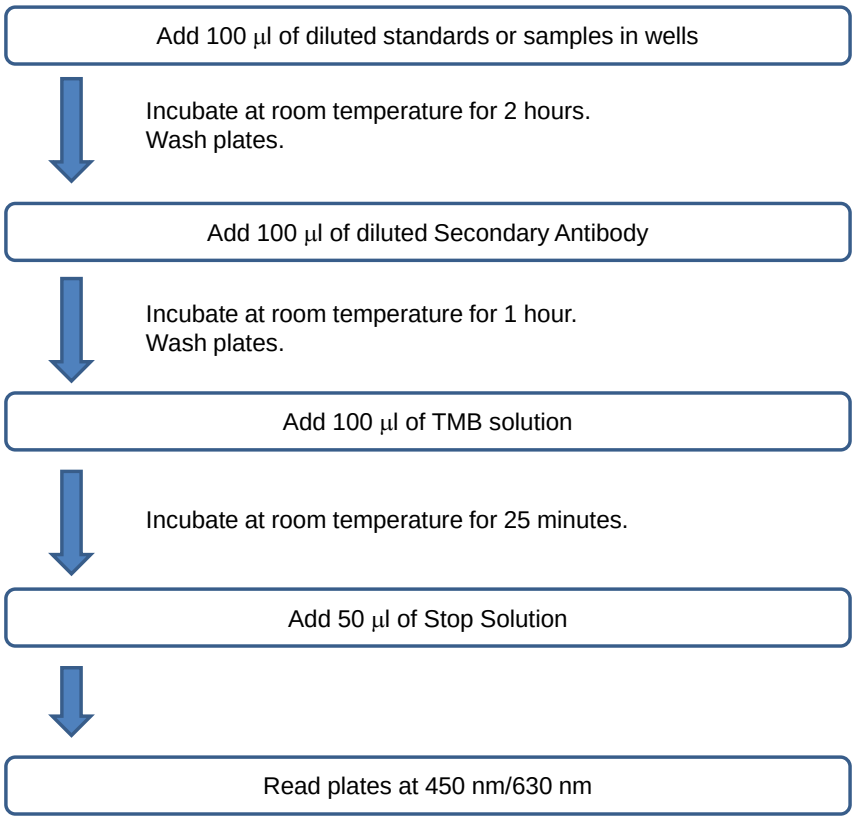


PLATE MAPPING

Example of the Mouse Anti-Gliadin IgG, G2a and G2b Antibody ELISA Kits

	1	2	3	4	5	6	7	8	9	10	11	12
A	10	10	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	5	5	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	2.5	2.5	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	1.25	1.25	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	0.63	0.63	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	0.31	0.31	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	0.16	0.16	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

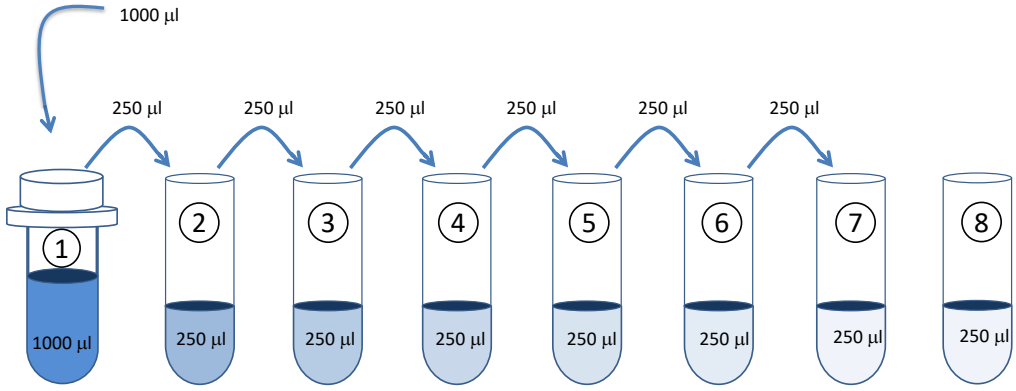
Example of the Mouse Anti-Gliadin IgG1 and M Antibody ELISA Kits

	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	25	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 PROCEDURE

1. **Prepare Standard Dilutions**: Please see the figure below for each assay's recommended standard range. Dissolve one vial of Standard in 1 ml of Sample/Standard/Secondary Antibody Dilution Buffer (Solution C) and keep it as a standard stock. Dilute the standard stock accordingly to get the first stock solution. Then serially dilute it with Solution C. For example, mix 250 µl of the first stock solution with an equal volume of Solution C to make the second stock solution, and then repeat it five more times. The original standard stock solution may be stored at -20ºC for use in a future assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



IgG/ IgG2a/ IgG2b:	10 ng/ml	5 ng/ml	2.5 ng/ml	1.25 ng/ml	0.63 ng/ml	0.31 ng/ml	0.16 ng/ml	Blank
IgG1/IgM:	50 ng/ml	25 ng/ml	12.5 ng/ml	6.3 ng/ml	3.1 ng/ml	1.6 ng/ml	0.8 ng/ml	Blank

2. **Prepare Sample Dilutions**: The dilution of serum from mice immunized with gliadin varies (1:1000 or more) depending on the immunization schedule and timing of serum collection. In general, no antibodies against OVA are observed in normal serum at a 1:1000 dilution.

   NOTE**:** Because regular mouse chows may contain gluten, unimmunized, naive mice may have anti-gliadin antibodies in serum.

3. **Add Standards and Samples**: Add 100 µl of standards, Solution C (blank), and samples to wells in duplicate. Incubate at room temperature for 2 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 Secondary Antibody**: Dilute one vial of Secondary Antibody in 10 ml Sample/Standard/Secondary Antibody Dilution Buffer (Solution C). Add 100 µl of secondary antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	2nd Antibody (µl)	Solution C (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

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 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

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

9. **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

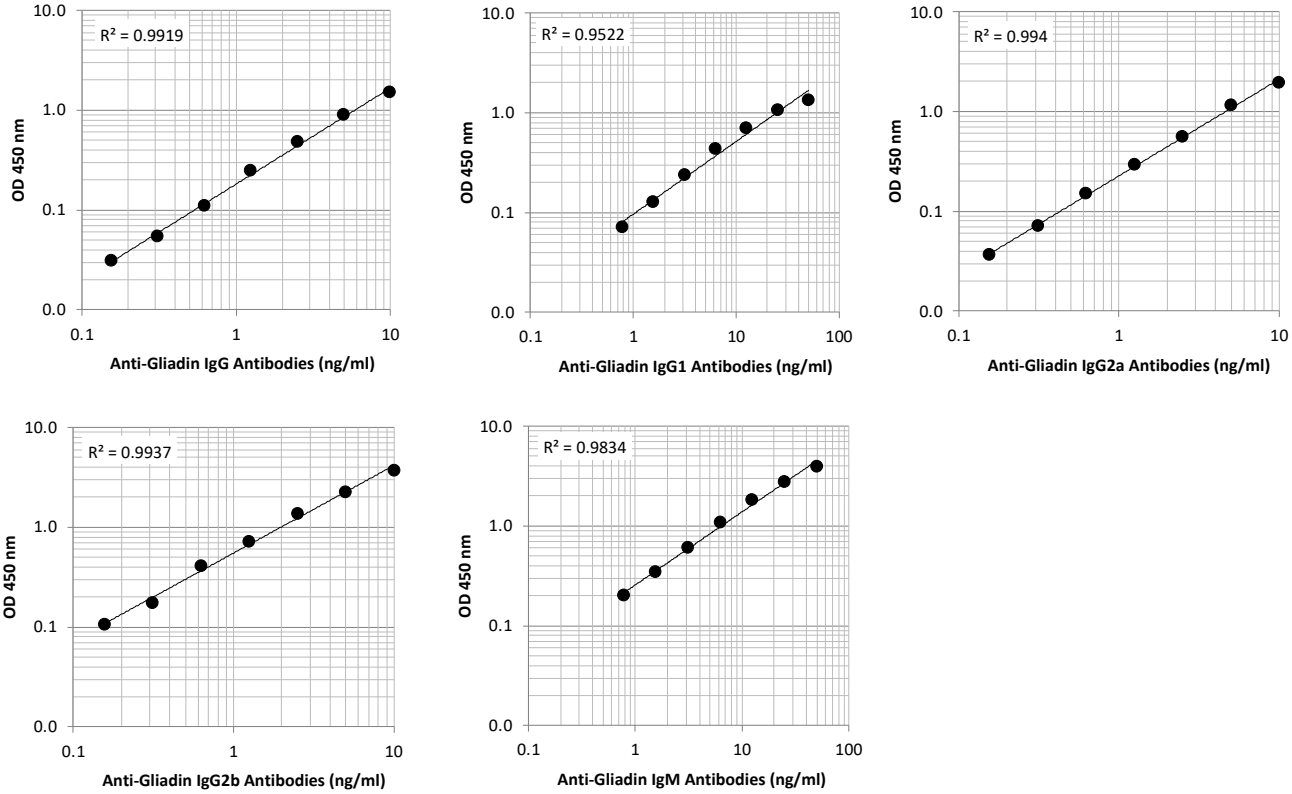
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

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-Gliadin Ig 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 original test samples.

Figure 1 - Typical Standard Curves for the Anti-Gliadin Antibody ELISA Kits



VALIDATION DATA

Table 1 - Reproducibility Data for the Mouse Anti-Gliadin IgG Antibody ELISA Kit

Test	0.31 ng/ml	1.25 ng/ml	5 ng/ml
Intra-Assay CV (%)	2.2	7.7	5.0
Inter-Assay CV (%)	8.6	7.1	5.5
Spike Test* (%)	101%	99%	109%

Table 2 - Reproducibility Data for the Mouse Anti-Gliadin IgG1 Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	8.2	8.4	3.0
Inter-Assay CV (%)	7.7	8.7	6.8
Spike Test* (%)	106%	104%	109%

Table 3 - Reproducibility Data for the Mouse Anti-Gliadin IgG2a Antibody ELISA Kit

Test	0.31 ng/ml	1.25 ng/ml	5 ng/ml
Intra-Assay CV (%)	4.8	3.5	4.0
Inter-Assay CV (%)	2.9	4.5	1.8
Spike Test* (%)	96%	106%	106%

Table 4 - Reproducibility Data for the Mouse Anti-Gliadin IgG2b Antibody ELISA Kit

Test	0.31 ng/ml	1.25 ng/ml	5 ng/ml
Intra-Assay CV (%)	3.8	4.1	1.8
Inter-Assay CV (%)	6.0	4.0	8.3
Spike Test* (%)	105%	105%	108%

Table 5 - Reproducibility Data for the Mouse Anti-Gliadin IgM Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	2.5	3.2	3.4
Inter-Assay CV (%)	4.9	4.8	2.3
Spike Test* (%)	98%	97%	101%

*Known amounts of anti-gliadin antibodies were added to samples and then diluted with Sample/Standard/Secondary Antibody Dilution Buffer to assay anti-gliadin 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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