Gliadin Detection ELISA Kit

Catalog # 6035

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify gliadin
FORMAT:	Precoated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4 hours
STANDARD RANGE:	20 – 0.31 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Liquid samples and biological fluids (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	1:5 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (5.6–6.1%)/Inter-Assay (3.4-8.1%)/Spiking Test (105-109%)
NOTES:	Gliadin in samples must be solubilized before starting assays (please see Sample Preparation below)

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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).

Gluten-related disorders can only be treated by life-long wheat exclusion diets (3, 4). These diets must consist only of "gluten-free" products, which are defined as containing up to 20 mg gluten per kg of raw or cooked food, (5–9). Therefore, precise gluten monitoring systems must be used to keep gluten levels below the acceptable threshold that defines "gluten free" limits in food (10). One such system, sandwich ELISAs are widely used in the food industry, however, flaws exist that can affect assay reliability such as allergen size limitations, sample processing (denatured protein structures due to heating and hydrolyzing processes) and non-validated extraction protocols (gliadin is difficult to solubilize and extract). Standardized assay systems demonstrating better accuracy and sensitivities are thus needed in the food industry.

To evaluate gliadin levels in samples, Chondrex, Inc. offers a Gliadin Detection Kit (Catalog # 6035) which employs two monoclonal antibodies recognizing a repeating epitope among gliadins and demonstrates high assay sensitivity. Please visit www.chondrex.com for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Gliadin (60351)	1 vial	20 ng, lyophilized	-20°C
Anti-Gliadin Detection Antibody (60353)	1 vial	100 µl	-20°C
Solution C - Sample/Standard/Secondary Antibody Dilution Buffer (30106)	1 bottle	50 ml	-20°C
Solution E - Detection Antibody Dilution Buffer (60356)	1 bottle	10 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	1 bottle	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-Gliadin Antibody Coated ELISA Plate (Silver)	1 each	96-well (8-well strips x 12)	-20°C

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ASSAY OUTLINE

Add 100 µl of diluted standards or samples in wells

↓ Incubate at room temperature for 2 hours.
Wash plates.

Add 100 µl of diluted Anti-Gliadin Detection Antibody

↓ Incubate at room temperature for 1 hour.
Wash plates.

Add 100 µl of diluted Streptavidin Peroxidase

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

Add 100 µl of TMB solution

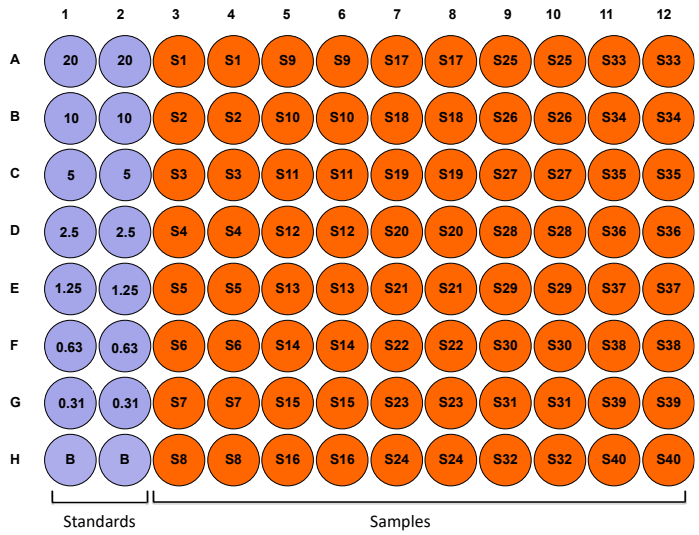
↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

PLATE MAPPING



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SAMPLE PREPARATION (*11*, *12*)

REQUIRED MATERIALS (NOT INCLUDED)

1. Dithiothreitol (DTT) (Alternatively 2-Mercaptoethanol (2-ME) can be used)
2. 1-isopropanol (IPA) (Alternatively Isopropa-2-nol or ethyl alcohol can be used)

PROCEDURE

1. Suspend 5-50 mg samples in 1 ml of 40% (v/v) IPA containing 1% (w/v) DTT (75mM) in water by vortex mixing.
2. Incubate the suspension at 50 °C for 60 minutes under continuous agitation.
3. Centrifuge for 10 minutes at 20,800 x g and collect supernatant.
4. Dilute the supernatant at 1:5 or higher with Solution C for subsequent analysis.

NOTE: The expected highest possible gliadin concentration after extraction is 500 µg/ml due to its lower solubility. Depending on the sample types, use an appropriate sample amount (mg) to make the initial suspension. For more information about gliadin sample preparation, please contact support@chondrex.com.

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.

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 0.31 - 20 ng/ml. Dissolve one vial of Standard (20 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 20 ng/ml solution with an equal volume of Solution C to make a 10 ng/ml solution, and then repeat it five more times for 5. 2.5, 1.25, 0.63, and 0.31 ng/ml standard solutions.

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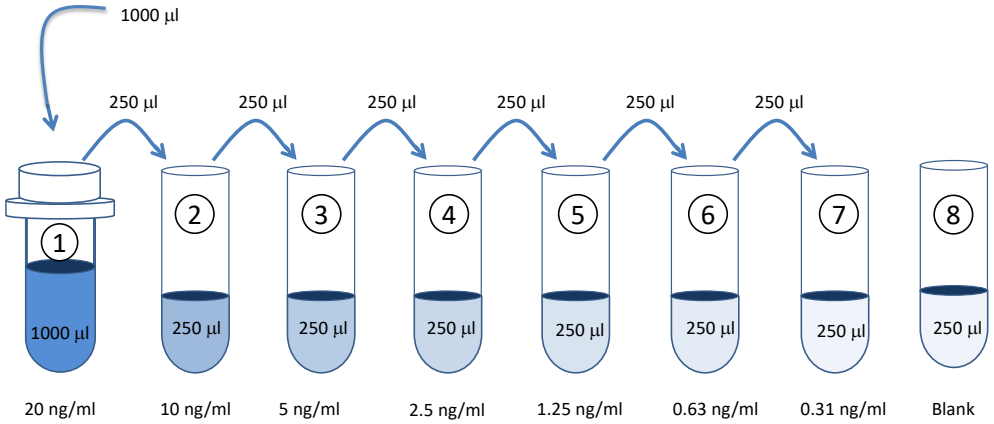

2. **Prepare Sample Dilutions**: Dilute samples at least 1:5 with Solution C depending on the estimated gliadin level in the samples. Two or three different sample dilutions are recommended if the gliadin levels in the samples are unknown.

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 Gliadin Detection Antibody**: Dilute one vial of Anti-Gliadin Detection Antibody in 10 ml Detection Antibody Dilution Buffer (Solution E). Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Gliadin Detection (µl)	Solution E (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

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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

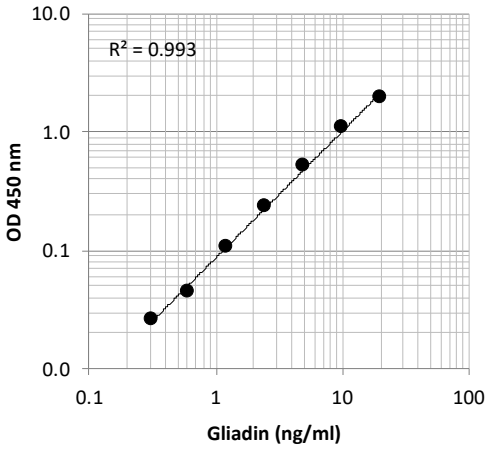
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

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 where the standard range is 0.31 to 20 ng/ml.

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 Gliadin Detection ELISA Kit



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VALIDATION DATA

Table 1 - Reproducibility Data for the Gliadin Detection ELISA Kit

Test	0.6 ng/ml	2.5 ng/ml	10 ng/ml
Intra-Assay CV (%)	1.3	3.2	1.1
Inter-Assay CV (%)	4.1	8.9	4.1
Spike Test* (%)	100%	101%	101%

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