Glutenin Detection ELISA Kit

Catalog # 6052

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify glutenin
FORMAT:	Precoated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	5 hours
STANDARD RANGE:	200 – 3.1 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 (1.9-8.3%)/Inter-Assay (2.1-7.0%)/Spiking Test (91-102%)
NOTES:	Glutenin 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 (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. 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).

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 (glutenin is difficult to solubilize and extract). Standardized assay systems demonstrating better accuracy and sensitivities are thus needed in the food industry.

To evaluate glutenin levels in samples, Chondrex, Inc. offers a Glutenin Detection Kit (Cat # 6052) which employs two monoclonal antibodies and demonstrates high assay sensitivity. Chondrex, Inc. also provides a Gliadin Detection Kit (Cat # 6035) for further fraction analysis. Please visit www.chondrex.com for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Glutenin (60521)	1 vial	200 ng, lyophilized	-20°C
Anti-Glutenin Detection Antibody (60523)	1 vial	100 µl	-20°C
Solution C - Secondary Antibody Dilution Buffer (2073)	1 bottle	10 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	3 bottles	20 ml	-20°C
Streptavidin Peroxidase (90291)	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-Glutenin Antibody Coated ELISA Plate (Brown)	1 each	96-well (8-well strips x 12)	-20°C

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SAMPLE PREPARATION (*11*)

REQUIRED MATERIALS (NOT INCLUDED)

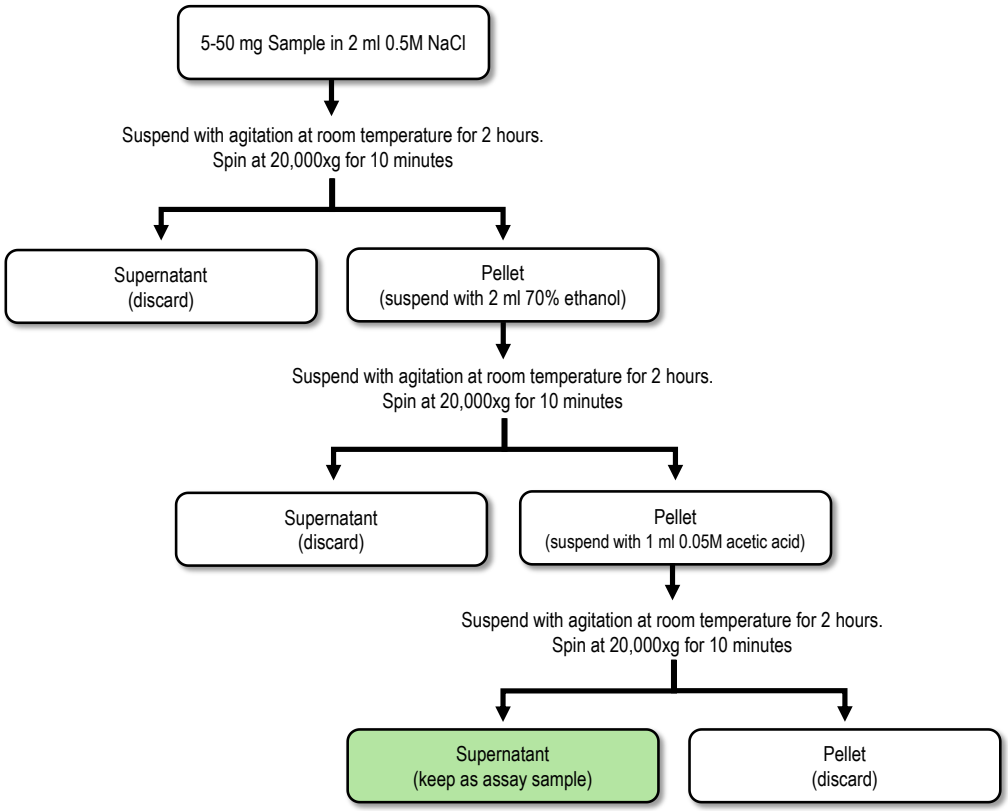
1. 0.5M NaCl
2. 0.05M Acetic Acid (Cat # 9075)
3. 70% Ethanol

PROCEDURE

1. Suspend 5-50 mg samples in 2 ml of 0.5M NaCl and incubate at room temperature for 2 hours under continuous agitation.
2. Centrifuge for 10 minutes at 20,000 x g and collect pellet.
3. Suspend pellet in 2 ml of 70% Ethanol and incubate at room temperature for 2 hours under continuous agitation.
4. Centrifuge for 10 minutes at 20,000 x g and collect pellet.
5. Suspend pellet in 1 ml of 0.05M acetic acid and incubate at room temperature for 2 hours under continuous agitation.
6. Centrifuge for 10 minutes at 20,000 x g and collect SUPERNATANT. This is your assay sample.
7. Dilute the supernatant at 1:5 or higher with assay buffer Solution D (Cat # 9055) for subsequent analysis.

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

Figure 1 - Sample Preparation Flowchart



ASSAY OUTLINE

Add 100 µl of Solution D to all wells

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

Add 100 µl of diluted standards and samples

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

Add 100 µl of diluted 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

	1	2	3	4	5	6	7	8	9	10	11	12
A	200	200	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	100	100	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	50	50	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	25	25	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	12.5	12.5	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	6.3	6.3	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	3.1	3.1	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

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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.

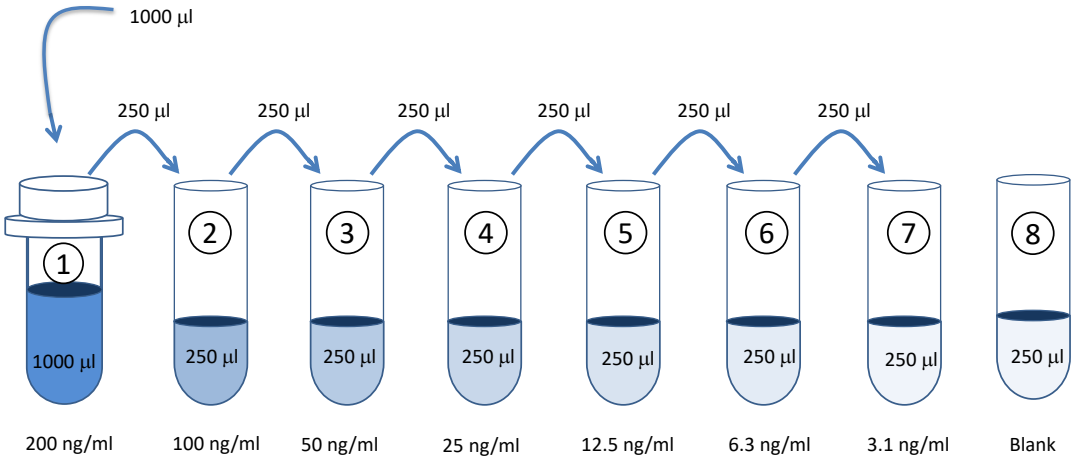
NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used 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.

ASSAY PROCEDURE

1. **Add Blocking Buffer:** Add 100 µl of Streptavidin Peroxidase Dilution Buffer (Solution D) to all wells. Incubate for 1 hour at room temperature.

2. **Prepare Standard Dilutions**: The recommended standard range is 3.1 - 200 ng/ml. Dissolve one vial of Standard (200 ng/vial) in 1 ml of Streptavidin Peroxidase Dilution Buffer (Solution D) and keep it as a standard stock. Then serially dilute it with Solution D. For example, mix 250 µl of the 200 ng/ml solution with an equal volume of Solution D to make a 100 ng/ml solution, and then repeat it five more times for 50. 25, 12.5, 6.3, and 3.1 ng/ml standard solutions.



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

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 Standards and Samples**: Add 100 µl of standards, Solution D (blank), and samples to wells in duplicate. Incubate at room temperature for 2 hours.

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 Detection Antibody**: Dilute one vial of Anti-Glutenin Detection Antibody in 10 ml of Secondary Antibody Dilution Buffer (Cat # 2073) (Solution C). Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Detection Antibody (µl)	Solution C (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

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 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

10. **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.*

11. **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

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

13. **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

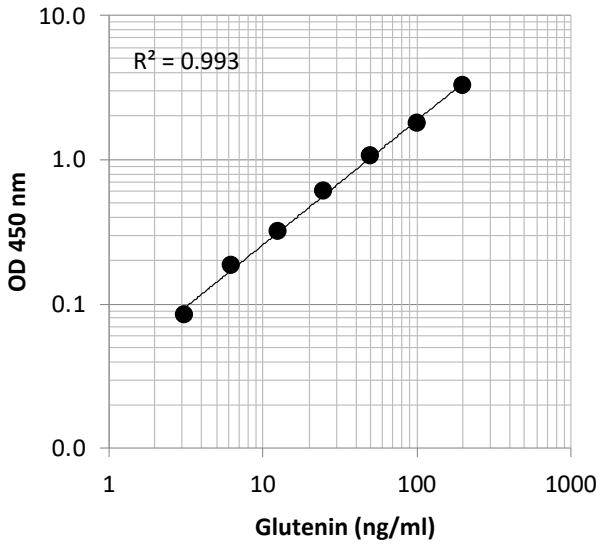
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

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 2 shows an example of a standard curve where the standard range is 3.1 to 200 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 2 - A Typical Standard Curve for the Glutenin Detection ELISA Kit



VALIDATION DATA

Table 1 - Reproducibility Data for the Glutenin Detection ELISA Kit

Test	6.3 ng/ml	25 ng/ml	100 ng/ml
Intra-Assay CV (%)	4.8	1.9	8.3
Inter-Assay CV (%)	2.1	7.0	4.6
Spike Test* (%)	102%	99%	91%

*Known amounts of glutenin were added to samples and then diluted with Solution D to assay glutenin by ELISA.

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TROUBLESHOOTING

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

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