## PRECAUTIONS

1. Ethanol solution is highly flammable. Keep container tightly closed, and keep away from heat, sparks, open flame and those smoking. It is toxic if swallowed, or if vapor is inhaled. Avoid contact with skin.
2. Components of Alert for Gliadin R5, such as controls and extraction additive, may contain one or more of the following potentially allergic materials: gluten, casein, almond protein and soy protein. If allergic to any of these compounds, use caution when using this product.
3. Store test kit between 2–8°C (35–46°F) when not in use. Do not freeze test kits, and avoid prolonged storage at ambient temperatures.
4. Bring kits to room temperature (18–30°C, 64–86°F) prior to use.
5. Do not use kit components beyond expiration date.
6. Do not mix reagents from one kit serial with reagents from a different kit serial.
7. Do not run more than 6 wells per test.
8. Use only incubation times specified; others may give inaccurate results.

## PROCEDURAL NOTES

1. **Sample extract dilution solution (PBS).** Prepare extract dilution solution by adding a foil pouch of dilution solvent, 10 mM PBS, to 1 L distilled or deionized water. Swirl to mix thoroughly.
2. **Wash buffer.** Wash buffer. Prepare the wash buffer solution by pouring the wash buffer concentrate into a 1 L container. Add 960 mL of distilled or deionized water. Swirl to assure thorough mixing.
   **Note:** Discard unused portions of extraction solution and wash buffer when the test kit has been used completely.
3. **Extraction additive.** Use extraction additive with all the samples utilizing procedure A or B (non-heat processed samples). Heat processed samples (procedure C) should **only** use the extraction additive if samples contain buckwheat, chestnut flour or tannins/phenolic compounds like chocolate, coffee, cocoa, wine, herbs or fruits.
4. **Antibody wells.** Keep wells sealed in the foil pouch until needed. Remove wells from the foil pouch only after samples are extracted, and the test procedure is set to begin.

## SAMPLE PREPARATION AND EXTRACTION

For analyzing **heat processed samples**, follow extraction **procedure C**. For all commodities that were **not heat processed**, follow either extraction procedure **A or B**. Samples of an unknown origin should be extracted using extraction procedure **C**.

### A. Extraction of non-heat processed samples with orbital shaker or rotator

1. Prepare 60% ethanol extraction solution by combining 6 parts ethanol with 4 parts distilled water.
2. Add 1 g ground sample, or 1 mL liquid sample, to a clean 50 cc tube.
3. Add 1 level scoop of extraction additive to the tube.
4. Add 10 mL (9 mL for liquid samples) of 60% ethanol to the tube, cap tightly, then shake the tube vigorously by hand for about 1 minute, or vortex for 30 seconds, to ensure complete mixing.
5. Extract by shaking (150 rpm) in an orbital shaker or rotator by laying down the tube on its side over the flat pad of the instrument, and holding it tightly using a rubber band or tape. Rotate or shake for 10 minutes at room temperature.
6. Remove the tube and let it stand in a rack for about 10 minutes to enable the sample extract to settle before withdrawing the clear extract. If necessary, centrifuge sample for 10 minutes at ≥ 2,500 g at room temperature.
7. Dilute each sample 1:50 by withdrawing 100 µL of the upper layer of the extract and transferring it to a prefilled dilution dropper bottle containing 4.9 mL of PBS. (Cap and tip must be removed first, then reinserted).
8. To mix, invert several times by hand or vortex for 5 seconds.
9. Test diluted samples within 2–3 hours of extraction.

### B. Extraction of non-heat processed samples with shaker or shaker water bath

1. Prepare 60% ethanol extraction solution by combining 6 parts ethanol with 4 parts distilled water.
2. Add 2 g ground sample, or 2 mL liquid sample, to a 125 mL clean extraction bottle.
3. Add 1 level scoop of extraction additive to the bottle.
4. Add 20 mL (18 mL for liquid samples) of 60% ethanol, cap the bottle tightly, then shake vigorously by hand for about 20 seconds to ensure complete mixing.
5. Extract by shaking (150 rpm) in a shaker for 10 minutes at room temperature (a shaker water bath can work, but do not turn the heat on). Remove the bottle from shaker or bath.
6. Let the bottle stand for about 10 minutes to enable some of the sample to settle before withdrawing the clear extract. If necessary, centrifuge sample for 10 minutes at ≥ 2,500 g at room temperature.
7. Dilute each sample 1:50 by withdrawing 100 µL of the upper layer of the extract and transferring it to a prefilled dilution dropper bottle containing 4.9 mL of PBS. (Cap and tip must be removed first, then reinserted.)
8. To mix, invert several times by hand or vortex for 5 seconds.
9. Test diluted samples within 2–3 hours of extraction.

### C. Extraction of heat processed commodities

Heat processed commodities require the gliadin renaturing cocktail solution (Neogen item #8515) that re-natures the heated sample and allows the proper determination of the gliadin in the sample. Prefilled dilution bottles **cannot** be used for this extraction procedure.

1. Prepare 80% ethanol extraction solution by combining 8 parts ethanol with 2 parts distilled water.
2. Prepare sample extract dilution solution (PBS) as detailed in procedural note #1.
3. Weigh out 0.25 g sample to 50 cc screw cap centrifuge tube.
4. Add 2.5 mL of renaturing cocktail solution.
5. If samples contain buckwheat, chestnut flour or tannins/phenolic compounds like chocolate, coffee, cocoa, wine, herbs or fruits, add one level scoop of extraction additive. For all other commodity types, do not add extraction additive.
6. Cap and vortex 30 seconds to homogenize cocktail and sample.
7. Incubate 40 minutes at 50°C (water bath or oven).
8. Remove samples and let cool for 5–10 minutes.
9. Add 7.5 mL of 80% ethanol and vortex again for 10–20 seconds.
10. Shake (150–200 rpm) for 1 hour at room temperature on a rotator (tube on its side).
11. Centrifuge sample (if necessary) for 10 minutes at ≥ 2,500 g at room temperature.
12. Dilute the sample 1:12.5 into 10mM PBS, pH 7.4 (200 µL sample into 2.3 mL PBS)
13. Samples are ready to run.

## TEST PROCEDURE

Allow the test kit and all reagents to warm to room temperature (18–30°C, 64–86°F) before using.

1. Remove 1 well for each sample to be tested plus 1 well for the control, and place into the well holder.
2. Mix each reagent by swirling its dropper bottle prior to use.
3. Add 3 drops from the yellow-labeled control dropper bottle to the first well. Add three drops from each diluted sample dropper bottle to a respective well as indicated in the template below. Mix for 20 seconds by sliding the microwell holder back and forth on a flat surface.

   Control S1 S2 S3 S4 S5

4. Incubate microwells **10 minutes** at room temperature (18–30°C, 64–86°F).
5. Shake out the contents of the wells. Using a wash bottle filled with wash buffer, fill each well and shake out. Repeat 5 times. Remove excess wash buffer by turning wells upside down and vigorously tapping wells on absorbent towel.
6. Add 3 drops from the blue-labeled conjugate dropper bottle to each well. Mix for 20 seconds by sliding the microwell holder back and forth on a flat surface.
7. Incubate for **10 minutes** at room temperature (18–30°C, 64–86°F).
8. Shake out the contents of the wells. Using a wash bottle filled with wash buffer, fill each well and shake out. Repeat 5 times. Remove excess wash buffer by turning wells upside down and vigorously tapping wells on absorbent towel.
9. Add 3 drops from the green-labeled substrate dropper bottle to each well. Mix for 20 seconds by sliding the microwell holder back and forth on a flat surface.
10. Incubate for **10 minutes** at room temperature (18–30°C, 64–86°F).
11. Add 3 drops from the red-labeled Red Stop dropper bottle to each well. Mix for 20 seconds by sliding the microwell holder back and forth on a flat surface. The results are now ready to be interpreted.

## INTERPRETATION OF RESULTS

Visually compare the color of a sample well to the color of the control well. If the sample well has **more blue color** than the control well, the sample tests positive for gliadin contamination of **more than 10 ppm**. If the sample well has **less blue color, or more red color**, than the control well, the sample contains **less than 10 ppm** of gliadin contamination. **Note:** Standard controls were made from wheat gliadin and calculated as gliadin. Approximately 50% of the gluten is available as gliadin. Therefore, 10 ppm gliadin is equal to 20 ppm gluten.

**Alternative:** Read wells (wipe bottom of wells first) in a microwell reader with a 650 nm filter. If the sample well has an optical density (OD) higher than the control well, the sample is positive for gliadin contamination of more than 10 ppm. If the sample well has an OD lower than the control well, the sample contains less than 10 ppm of gliadin contamination.

## CUSTOMER SERVICE

Neogen Customer Assistance and Technical Service can be reached between 8 a.m. and 7 p.m. Eastern time by calling 800/234-5333 or 517/372-9200 and asking for a Neogen sales representative or Technical Services. Assistance is available on a 24-hour basis by calling 800/234-5333. Training on this product, and all Neogen test kits, is available.

**MSDS INFORMATION AVAILABLE**
Material safety data sheets (MSDS) are available for this test kit, and all of Neogen's test kits, on Neogen's Web site at www.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

**WARRANTY**
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16269A A-Gliadin-R5-0311



*Read instructions carefully before starting test*

# ALERT®

# Screening Test for Gliadin R5

*REFRIGERATE at 2–8°C (35–46°F) • DO NOT FREEZE*

**GLIADIN**
Gliadin is an alcohol-soluble protein found in wheat that belongs to a group of proteins called prolamins. Other prolamins include secalin, found in rye, and hordein, found in barley. Gluten consists of two groups of proteins (prolamins and glutelins) that are found in differing amounts in wheat, barley, rye and oats.

Gliadin and other prolamins have been identified as major causal agents in a number of disorders, including wheat allergy and gluten intolerance (celiac disease). Wheat allergy is a specific immune response to a number of wheat proteins, including gliadin, albumin, globulin, and glutenin. Celiac disease is a chronic reaction to gluten proteins that results in the poor absorption of nutrients in the small intestine.

Those who must avoid gluten rely upon the correct labeling of food to make appropriate, safe food choices. Testing for the presence of gluten components ensures food manufacturers that an unlabeled—and potentially dangerous—ingredient did not make its way into a food product.

**INTENDED USE**
Alert for Gliadin R5 is intended for the qualitative analysis of ingredients, clean-in-place solutions, finished food products, and environmental surfaces intended to be gluten-free for the presence of gliadin and prolamins found in wheat, rye and barley.

**INTENDED USER**
This test kit is designed for use by quality control personnel and others familiar with foods possibly contaminated by gluten. Since technique is very important, operators should be trained by a Neogen representative or someone who has completed the Neogen training.

**ASSAY PRINCIPLES**
Alert for Gliadin R5 is a sandwich enzyme-linked immunosorbent assay (S-ELISA). Gliadin is extracted from samples with a 60% ethanol solution by shaking in a shaker or rotator. Extract is diluted in phosphate saline buffer and diluted samples are added to antibody-coated wells (capture antibody) where gliadin will bind to the antibody during an incubation period. Any unbound gliadin is washed away and a second antibody, which is R5 enzyme labeled (detector antibody) is added. The detector antibody binds to the gliadin during another incubation period. Unbound enzyme-labeled antibody is washed away and a one step substrate is added. Color develops as a result of the presence of bound-labeled antibody. A stopping reagent is added and the color of the solution is observed. Blue color indicates samples containing high levels of gliadin while purple or red samples contain little or no gliadin.

**STORAGE REQUIREMENTS**
The kit can be used until the expiration date on the label when stored refrigerated at 2–8°C (35–46°F).

**MATERIALS PROVIDED**
1. 24 antibody-coated microwells
2. 1 yellow-labeled dropper bottle of 10 ppm gliadin control (20 ng/mL gliadin)
3. 1 blue-labeled dropper bottle of conjugate
4. 1 green-labeled dropper bottle of K-Blue® Substrate
5. 1 red-labeled dropper bottle of Red Stop solution
6. 20 prefilled dilution dropper bottles of 4.9 mL PBS
7. 1 foil pouch containing enough 10 mM PBS dry powder to prepare 1 L of dilution solvent
8. 1 wide-mouth bottle containing enough 10 mM PBS-Tween wash buffer concentrate to prepare 1 L in distilled or deionized water (pH 7.4)
9. 30 g of extraction additive in a specimen cup
10. Plastic 1 g scoop to measure extraction additive

**MATERIALS RECOMMENDED BUT NOT PROVIDED**
1. Orbital rotator or shaker to hold 50 cc centrifuge tubes for a 1 g sample or shaker or shaker water bath with clamps adjusted to hold 125 mL (4 oz) extraction bottles for a 2 g sample
2. Allergen Environmental Swabbing Kit (Neogen item #8432S)
3. Scale capable of weighing 1 ± 0.1 g (Neogen item #9427), or scale capable of weighing 0.25 g ± 0.01 g if using renaturing cocktail solution
4. Gliadin renaturing cocktail solution for analysis of heat processed samples (Neogen item #8515, 8515S, or 8515B)
5. 2 1 L bottles to prepare washing solution, extract solution (Neogen item #9472)
6. Timer (Neogen item #9426)
7. Microwell holder (Neogen item #9402)
8. Pipettor, 100 µL (Neogen item #9272, 9278, or 9276)
9. Pipette tips (Neogen item #9410)
10. Wash bottle (Neogen item #9400)
11. Paper towels or equivalent absorbent material
12. Waterproof marker
13. Distilled or deionized water
14. Laboratory grade ethanol (190 proof)
15. Centrifuge (optional)