AgraStrip® Gluten G12

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 061403

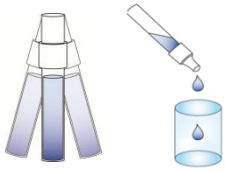
ROMER Romer Labs®

Order #: COKAL0200AS

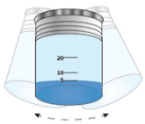
Gluten

Gluten is the main group of proteins in grains and consists of prolamins (in wheat: gliadin) and glutelins (in wheat: glutenins) occurring in the same ratio. Due to its physicochemical characteristics, gluten is used in food products as a binder. Coeliac disease is an autoimmune disorder of the small intestine. It is caused by a reaction to gliadin and the only effective treatment is a lifelong gluten-free diet. Due to Codex Standard 118-1979, "gluten-free" products must comply with gluten levels (including prolamin fractions from wheat, rye, barley and oats) below 20 mg/kg and "foods specially processed to reduce gluten content" must comply with levels between 20 and 100 mg/kg.

Short instructions



Perform extraction and dilution steps for finished product, rinse water or swab.



Shake dilution tube vigorously by hand for **15 seconds**.

Place a test strip vertically into the dilution tube and allow liquid to soak up the strip to the „flow" level line.

Then place the strip upright into a slot of the Tube Holder and allow to develop for **10 minutes** and **read off the result immediately**.

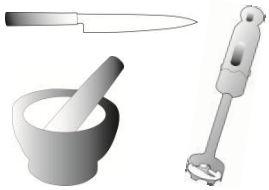
Performance Characteristics:

LOD: 5 – 10 – 20 ppm Gluten* (Finished product)
35 ppb Gluten* (Rinse water)
4 µg/25cm^2 Gluten** (Swab testing)

* LOD was determined in extraction solution
** LOD was calculated

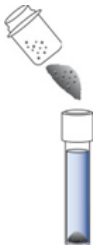
Sample preparation – Finished Products

1. Homogenize the sample (i.e. blend, crush, grind).

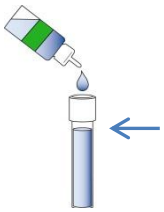


2. Weigh **0.2g** of sample (weigh with Balance or estimate by filling up one of the extraction tube caps).

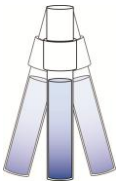
3. Add the sample to extraction tube



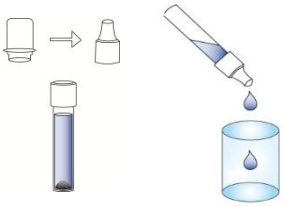
4. Fill Extraction Tube with ***Extraction Buffer*** to level shown below (blue arrow).



5. Close tube with tube cap and shake vigorously by hand for ***1 minute***.



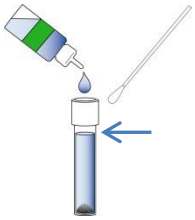
6. Remove cap from the extraction tube and replace with dropper tip. And transfer **3 drops** (**100µl**) to a dilution tube.



Proceed to the Assay Section (page 3) to complete your test

Sample preparation – Swab Testing

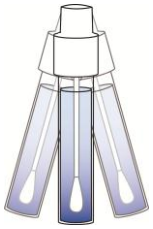
1. Fill extraction tube with **Extraction Buffer** to level shown below (blue arrow), take a swab and wet the end by dipping into the buffer



2. Wipe an area of ***5cmx5cm*** using side to side movements, rotating the swab tip as you go (we recommend the "cross-hatch" swabbing technique indicated below)

3. Place the swab into the extraction tube. Carefully break off the end at the pre-scored point.

4. Close the tube with a cap and shake vigorously for **1 minute**.



5. Remove cap from the extraction tube and replace with dropper tip. And transfer **3 drops** (**100µl**) to a dilution tube

Proceed to the Assay Section (page 3) to complete your test

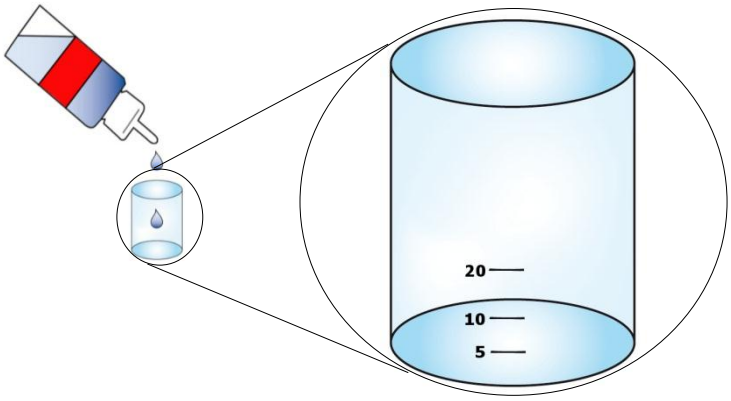
Sample preparation – Rinse water Testing

Add **0.5 mL** of rinse water into a dilution tube

Proceed to the Assay Section (page 3) to complete your test

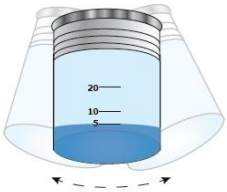
Assay Procedure in detail

1. Transfer various amounts of **Dilution Buffer** (red label) into the dilution tube already filled with extract

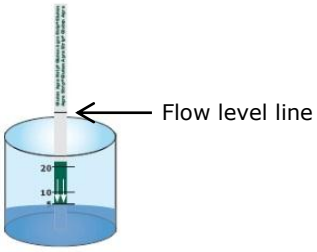


amount of dilution buffer	sample type
Fill up to ***20 mark***	Finished products with *20ppm cut off*
Fill up to ***10 mark***	Finished products with *10ppm cut off*
Fill up to ***5 mark***	Finished products with *5ppm cut off* Swab samples (cut off = 4 µg)
Add ***5 drops***	Rinse water samples

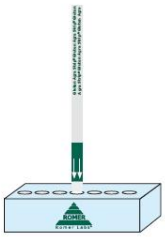
2. Close the dilution tube with the cap and shake vigorously by hand for **15 seconds**
3. Take off cap and place a test strip vertically (arrows pointing down) into the dilution tube and allow liquid to flow up the strip to the „flow" level line. (this takes about **45 seconds**)
4. After the liquid has soaked up to the "flow" level remove the test strip from the dilution tube and place it upright (arrows pointing down) into a slot of the Tube Holder and allow to develop for **10 minutes** and then read off the result immediately.



15 seconds

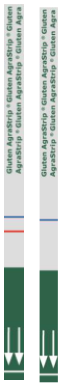


~45 seconds



10 minutes

Interpretation of Results



One single blue line (=control line) in the central part of the test = negative result

One red line (=test line) and one blue line in the result zone = positive result. The sample contains Gluten higher than the cutoff level and further investigations should be performed (e.g. quantification of Gluten using AgraQuant® Gluten G12 ELISA Test Kits).

No control line appears = invalid result, regardless of whether the test line appears. In the case of an invalid result, please repeat the procedure with a new strip. If the problem persists, please contact Romer Labs® before continuing further.

Important advice for the proper execution of the test:

It is important to ***read the results immediately after the 10 minute*** **incubate step** since the AgraStrip® test system has been validated extensively and shows reliable results after that exact time. Longer incubation times can lead to the development of false positive results.

Performance Characteristics in Detail

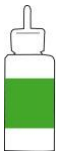
Limit of detection: 5 – 10 – 20 ppm Gluten (Finished product)
35 ppb Gluten (Rinse water)
4 μg/25cm^2 Gluten (Swab testing)

Range of detection: 5 – 10000 ppm Gluten

pH range: Performing the assay in a pH range of 6-8 will lead to reliable results. Highly acidic samples can lead to false positive results whereas in an alkaline milieu there is the tendency to false negative results.

Materials Supplied with Kit:

1 Tube containing 10 AgraStrip® Gluten G12 Strips

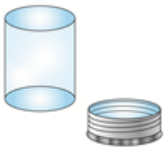


1 big Dropper Bottle (***green label***) containing 35mL **Extraction Buffer**

1 small Dropper Bottle containing 10mL **Dilution Buffer**

10 Extraction Tubes

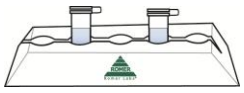
10 Tube Caps



10 Dilution Tubes with caps

10 Extraction Tube Dropper Tips

10 Breakpoint Swabs



1 Tube Holder

Materials required but not supplied for solid sample preparation

- Blender or Crusher or Blade

Technical and Background Information

The AgraStrip® Gluten G12 Test Kit is a lateral flow assay for the detection of Gluten content in food, rinse waters and environmental swab samples.

Gluten Allergy
Gluten is the main group of proteins in grains and consists of prolamins (in wheat: gliadin) and glutelins (in wheat: glutenins) occurring in the same ratio. Due to its physicochemical characteristics, gluten is used in food products as a binder. Coeliac disease is an autoimmune disorder of the small intestine. It is caused by a reaction to gliadin and the only effective treatment is a lifelong gluten-free diet. Due to Codex Standard 118-1979, "gluten-free" products must comply with gluten levels (including prolamin fractions from rye, barley and oats) below 20 mg/kg and "foods specially processed to reduce gluten content" must comply with levels between 20 and 100 mg/kg.

Assay Principles
The AgraStrip® Gluten G12 Test Kit is an immunochromatographic test for the detection of gluten in foodstuffs. The test kit uses a new monoclonal antibody called G12 that specifically recognises the pathogenic fragment of the gliadin protein present in gluten. This fragment is called 33-mer and triggers the auto-immune reaction in coeliac patients. During the test, the sample reacts with a coloured conjugate (anti-gliadin 33mer monoclonal antibody – red-coloured microsphere) which forms a complex with the reagent on the strip. This complex spreads along the membrane by capillary action. The AgraStrip® Gluten G12 is easy to use, fast and reliable.

Approvals
This test kit's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications. The following matrices have been validated according to AOAC Performance Tested Methods (PTM) protocols: rice flour, bread, cookies, ice cream and dark chocolate. Stainless steel has been validated per AOAC PTM protocol for environmental surface swab testing.

The rinse water test method has been validated internally by Romer Labs®.

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER 061403

Precautions

1. The product must be stored in its original package, between 15 and 25°C (room temperature). Do not use components beyond the expiration date indicated on the kit labels. Do not open the product until needed.
2. Test strips must be kept inside their original packaging, closed as tightly as possible. Do not freeze.
3. Adhere to the instructions for test procedures.
4. The components in this test kit have been quality control tested as a standard batch unit. Do not mix components from different lot numbers.

Sampling:
Consideration must be taken that the food may contain an uneven distribution of Gluten (spot contamination). It is important to test a representative portion of food as only a small amount of material is tested with the AgraStrip® Gluten test.

Detection:
The detection limit of the AgraStrip® Gluten G12 test is at the low ppm level but will vary depending on the food matrix being tested. To give reliable results each individual matrix should be validated before the kit is used routinely. Since the assay is for screening purpose a positive result might require confirmation or further testing.
For further information regarding validation please contact Romer Labs.

Note:
! Chocolate and flour samples may block the filter tip of the extraction tube. This can be avoided by transferring the extract directly from the extraction tube to the reaction vial using a pipette or by hand to a level just under the 0.5ml graduation of the reaction vial !

Handling of the new improved AgraStrip® extraction & dilution buffer bottles

To **open** the bottle just hold the neck of the upper part (indicated by the asterix) with your thumb and index finger and twist the corrugated screw top counterclockwise .

To **close** the bottle twist the screw top clockwise up to the stop.

For further information please contact:

COMPETENCE CENTER ALLERGENS: **Romer Labs UK Ltd.** **Block 5, The Heath Business &** **Technical Park,** **Runcorn, Cheshire, WA7 4QX**	**Tel:+44 (0) 845 519 50 10** **Email: enquiry@romerlabs.com**
GLOBAL HEAD QUARTERS: **Romer Labs Division Holding GmbH** **Technopark 1** **3430 Tulln, Austria**	**Tel: +43 (0) 2272 615 33** **Email : office-europe@romerlabs.com**

For customer service contact details in your country please visit:

www.romerlabs.com

Warranty

The user assumes all risk in using Romer Labs UK Ltd products and services. Romer Labs UK Ltd will warrant that its products and services meet all quality control standards set by Romer Labs UK Ltd, and Romer Labs UK Ltd will, at its option, repair or replace any product, components, or repeat services which prove to be defective in workmanship or material within product specific warranty periods or expiration dates and which our examination shall disclose to our satisfaction to be defective as such. This warranty is expressly in lieu of all other warranties, expressed or implied, as to description, quality, merchantability, fitness for any particular purpose, productiveness, or any other matter. Romer Labs UK Ltd shall be in no way responsible for the proper use of its products. Romer Labs UK Ltd hereby disclaims all other remedies, warranties, guarantees or liabilities, expressed or implied, arising by law or otherwise, and it shall have no liability for any lost profits or damage, direct, indirect or otherwise, to person or property, in connection with the use of any of its products or services. This warranty shall not be extended, altered or varied except by a written instrument signed by an authorized representative of Romer Labs UK Ltd

FOR NOTES