



# CERTIFICATION

## AOAC Research Institute *Performance Tested Methods*<sup>SM</sup>

Certificate No.  
**011601**

The AOAC Research Institute hereby certifies the method known as:

### Gluten Protein Rapid Kit

manufactured by

**Neogen Corporation**  
**620 Lesher Place**  
**Lansing, Michigan 48912**  
**USA**

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*<sup>SM</sup> Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, Senior Director  
Signature for AOAC Research Institute

Issue Date  
Expiration Date

December 05, 2024  
December 31, 2026

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| <b>AUTHORS</b><br><b>ORIGINAL VALIDATION:</b> Luke Emerson-Mason, Raquel Sobel, Adam Bouchard, James Boghosian, Thomas Grace<br><b>MODIFICATION SEPTEMBER 2022:</b> April Schumacher                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |  | <b>SUBMITTING COMPANY</b><br>Elution Technologies<br>480 Hercules Drive<br>Colchester, VT 05446                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <b>CURRENT SPONSOR</b><br>Neogen Food Safety Corporation<br>620 Leshner Place<br>Lansing, MI 48912 |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                    |
| <b>METHOD NAME</b><br>Neogen® Gluten Protein Rapid Kit<br>Formerly known as 3M™ Gluten Protein Rapid Kit and Elution Technologies Gluten Rapid Test Kit                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |  | <b>CATALOG NUMBER</b><br>L25GLU                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                    |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                    |
| <b>INDEPENDENT LABORATORY</b><br>Q Laboratories<br>1400 Harrison Ave.<br>Cincinnati, OH 45214 USA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                    |
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| <b>APPLICABILITY OF METHOD</b><br>Target analyte – Gluten.<br><br>Matrixes – Buckwheat (0.2 g), dry cereal (0.2 g), rice flour (0.2 g), incurred bread dough (0.2 g), pasteurized soy milk (100 µL), chocolate syrup (500 µL), CIP Clean in Place solution (200 µL), stainless steel (100 cm²)<br><br>Performance claims – The Gluten Protein Rapid Kit utilizes the Poly-G antibody to detect the toxic alpha- gliadin fragment of the gluten globulin compound from wheat, rye, barley, and their cultivars. The LOD for food products, including raw ingredients, finished products and CIP, is 5 ppm gluten, or 5 µg gluten per ml per 100 cm² swabbed surface area. Samples containing greater than 10 ppm gluten may show positive results in 5 minutes or less. |  | <b>REFERENCE METHOD</b><br><br>OMA 2012.01 Gliadin as a Measure of Gluten in Foods Containing Wheat, Rye, and Barley, Immer, U., Haas-Lauterbach, S., (2012) J. AOACI Vol. 95, No. 4 1118-1124 (4)                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                    |
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| <b>ORIGINAL CERTIFICATION DATE</b><br>January 20, 2016                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |  | <b>CERTIFICATION RENEWAL RECORD</b><br>Renewed through December 2026.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                    |
| <b>METHOD MODIFICATION RECORD</b><br><div><div>1. January 2018 Level 1</div><div>2. January 2019 Level 1</div><div>3. December 2019 Level 1</div><div>4. June 2020 Level 1</div><div>5. December 2020 Level 1</div><div>6. September 2022 Level 2</div><div>7. January 2024 Level 1</div></div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  | <b>SUMMARY OF MODIFICATION</b><br><div><div>1. Ownership changed from Elution Technologies to 3M Food Safety, update kit name and supporting documentation.</div><div>2. Conversion of inserts and labels to 3M formatting.</div><div>3. Editorial/clerical changes.</div><div>4. Editorial changes to accurately reflect upper limit as 10,000 ppm.</div><div>5. Addition of 2 general warning statements regarding allergens.</div><div>6. Manufacturing location change for bottling of buffer solutions and kit assembly.</div><div>7. Editorial changes to rebrand method from 3M to Neogen Corporation.</div></div> |                                                                                                    |
| Under this AOAC <i>Performance Tested Method</i> <sup>SM</sup> License Number, 011601 this method is distributed by:<br>NONE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |  | Under this AOAC <i>Performance Tested Method</i> <sup>SM</sup> License Number, 011601 this method is distributed as:<br>NONE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                    |

#### PRINCIPLE OF THE METHOD (1)

The Gluten Protein Rapid Kit is a rapid immunochromatographic lateral flow test device which utilizes a purified proprietary poly-clonal antibody developed by ICL against the alpha –gliadin fraction of gluten.

#### DISCUSSION OF THE VALIDATION STUDY (1)

This study demonstrates that the Gluten Protein Rapid Kit can detect gluten in a broad spectrum of matrixes, without cross-reactivity or interference; additionally, the assay has been shown to detect gluten in an incurred sample at 15 ppm, as well as detecting gluten spiked at high concentrations with no hook effect. Food matrix samples containing greater than 10 ppm gluten had a combined POD of 0.967 at 5 minutes with 95% CI of 0.93, 0.98, while food matrixes containing 5 ppm to 10 ppm gluten had a combined POD of 0.581 with 95% CI of 0.53, 0.63. Thus, if a sample reports positive in 5 minutes, it is possible that it contains less than 10 ppm gluten; however, it is probable that it contains >10 ppm.

There was good correlation between sponsor and independent labs for chocolate syrup and corn flakes spiked with PWG at fractional recovery levels, as well for both matrixes spiked at 5 ppm, 10 ppm and 20 ppm; chocolate syrup was also spiked with a secondary reference material, NIST Wheat Flour SRM 1567b, at both 3.5 ppm and 5 ppm with good correlation. Additionally, stainless steel swabbing results were similar for both labs, with a fractional recovery level of 2.5 µg/ml/100cm². While both sponsor and independent labs found a low fractional recovery level of 1 ppm for corn flakes, PODs for corn flakes spiked at 0 ppm were 0.00 for both labs indicating no false positives results in this matrix.

**Table 1. Food Matrix Study - Sponsor Lab (1)**

| Matrix           | Spike concentration ppm | Number of replicates | Number of positive results |          | POD     |           | 95% CI     | 95% CI     | Average results from AOAC OMA 2012.01 n=3 |
|------------------|-------------------------|----------------------|----------------------------|----------|---------|-----------|------------|------------|-------------------------------------------|
|                  |                         |                      | at 5min                    | at 11min | at 5min | at 11 min | at 5 min   | at 11 min  |                                           |
| Buckwheat        | 0                       | 30                   | 0                          | 0        | 0.00    | 0.00      | 0.00, 0.11 | 0.00, 0.11 | <3                                        |
|                  | 5                       | 30                   | 17                         | 30       | 0.57    | 1.00      | 0.39, 0.73 | 0.89, 1.00 | 5.73                                      |
|                  | 10                      | 30                   | 26                         | 30       | 0.87    | 1.00      | 0.70, 0.95 | 0.89, 1.00 | 9.4                                       |
|                  | 20                      | 30                   | 29                         | 30       | 0.97    | 1.00      | 0.83, 1.00 | 0.89, 1.00 | 15.13                                     |
| Chocolate Syrup  | 0                       | 30                   | 0                          | 0        | 0.00    | 0.00      | 0.00, 0.11 | 0.00, 0.11 | <3                                        |
|                  | 5                       | 30                   | 11                         | 30       | 0.37    | 1.00      | 0.22, 0.54 | 0.89, 1.00 | 4.7                                       |
|                  | 10                      | 30                   | 25                         | 30       | 0.83    | 1.00      | 0.66, 0.93 | 0.89, 1.00 | 9.17                                      |
|                  | 20                      | 30                   | 30                         | 30       | 1.00    | 1.00      | 0.89, 1.00 | 0.89, 1.00 | 15.1                                      |
| Soy Milk         | 0                       | 30                   | 0                          | 0        | 0.00    | 0.00      | 0.00, 0.11 | 0.00, 0.11 | <3                                        |
|                  | 5                       | 30                   | 18                         | 30       | 0.60    | 1.00      | 0.42, 0.75 | 0.89, 1.00 | 7                                         |
|                  | 10                      | 30                   | 29                         | 30       | 0.97    | 1.00      | 0.83, 1.00 | 0.89, 1.00 | 11.63                                     |
|                  | 20                      | 30                   | 30                         | 30       | 1.00    | 1.00      | 0.89, 1.00 | 0.89, 1.00 | 17.23                                     |
| Corn Flakes      | 0                       | 30                   | 0                          | 0        | 0.00    | 0.00      | 0.00, 0.11 | 0.00, 0.11 | <3                                        |
|                  | 5                       | 30                   | 26                         | 30       | 0.87    | 1.00      | 0.70, 0.95 | 0.89, 1.00 | 5                                         |
|                  | 10                      | 30                   | 30                         | 30       | 1.00    | 1.00      | 0.89, 1.00 | 0.89, 1.00 | 8.7                                       |
|                  | 20                      | 30                   | 30                         | 30       | 1.00    | 1.00      | 0.89, 1.00 | 0.89, 1.00 | 15.57                                     |
| White Rice Flour | 10,000                  | 10                   | 10                         | 10       | 1.00    | 1.00      | 0.72, 1.00 | 0.72, 1.00 | N/A                                       |

**Table 2. CIP Rinse Water Study (1)**

| Matrix | Spike concentration ppm | Number of replicates | Number of positive results |          | POD     |           | 95% CI     | 95% CI     | Average results from AOAC OMA 2012.01 n=3 |
|--------|-------------------------|----------------------|----------------------------|----------|---------|-----------|------------|------------|-------------------------------------------|
|        |                         |                      | at 5min                    | at 11min | at 5min | at 11 min | at 5 min   | at 11 min  |                                           |
| CIP    | 0                       | 30                   | 0                          | 0        | 0       | 0         | 0.00, 0.11 | 0.00, 0.11 | <3                                        |
|        | 5                       | 30                   | 30                         | 30       | 1       | 1         | 0.89, 1.00 | 0.89, 1.00 | 5.6                                       |
|        | 10                      | 30                   | 30                         | 30       | 1       | 1         | 0.89, 1.00 | 0.89, 1.00 | 9.1                                       |

**Table 3. Stainless Steel/ Swabbing Study Sponsor Lab (1)**

| Matrix          | Spike concentration (ug/ml/100cm2) | Number of replicates | Number of positive results |          | POD     |           | 95% CI     | 95% CI     |
|-----------------|------------------------------------|----------------------|----------------------------|----------|---------|-----------|------------|------------|
|                 |                                    |                      | at 5min                    | at 11min | at 5min | at 11 min | at 5 min   | at 11 min  |
| Stainless Steel | 0                                  | 5                    | 0                          | 0        | 0.00    | 0.00      | 0.00, 0.43 | 0.00, 0.43 |
|                 | 2.5                                | 30                   | 0                          | 15       | 0.00    | 0.50      | 0.00, 0.11 | 0.33, 0.67 |
|                 | 20                                 | 5                    | 5                          | 5        | 1.00    | 1.00      | 0.57, 1.00 | 0.57, 1.00 |

**DISCUSSION OF MODIFICATION APPROVED SEPTEMBER 2022 (3)**

Three lots of Gluten Protein Rapid Kit produced at a new manufacturing location was compared to one lot produced at the current manufacturing location. Acceptance criteria were based on the most current Gluten and Food Allergen Working Group draft recommendations for qualitative methods, as of September 2022. The Gluten Protein Rapid Kit method showed consistent product performance and met the acceptance criteria of a POD of 0.95 or greater at the CDC and a POD of 0.05 or less for a blank sample for all lots. The acceptance criteria for a blank sample were met when the results from all 3 lots were combined and when additional samples were tested for the third lot. Based on these results company recommends moving the Gluten Protein Rapid Kit to the new manufacturing location.

**REFERENCES CITED**

- Emerson-Mason, L., Sobel, R., Bouchard, A., Boghosian, J., and Grace, T., Evaluation of the 3M<sup>TM</sup> Gluten Protein Rapid Kit (formerly Elution Technologies Gluten Rapid Test) for the Detection for Gluten in Select Foods and Select Environmental Surfaces, AOAC *Performance Tested Method<sup>SM</sup>* certification number 011601.
- Immer, U., Haas-Lauterbach, S., (2012) J. AOACI Vol. 95, No. 4 1118-1124
- Schumacher, A., AOAC *Performance Tested Method<sup>SM</sup>* Level 2 Modification 3M<sup>TM</sup> Milk Protein Rapid Kit (Casein) - PTM 091701, 3M<sup>TM</sup> Coconut Protein Rapid Kit - PTM 061903, 3M<sup>TM</sup> Gluten Protein Rapid Kit - PTM 011601, and 3M<sup>TM</sup> Egg White Protein Rapid Kit - PTM 052001, Certification number 011601. Approved September 16, 2022.