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Gluten Free Test Lateral Flow kit

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Gluten Free Test Lateral Flow kit

1. Introduction

Gluten consists of two groups of proteins, namely the glutenins and the prolamins. Prolamins are present in wheat (gliadin), barley (hordein) and rye (secalin), and are the alcohol-soluble fraction of gluten. Consumption of gluten might be harmful for people with gluten intolerance (celiac disease) and wheat allergy, because the small intestine is unable to absorb nutrients. It might cause a broad range of symptoms, such as nausea, vomiting, abdominal pain, joint pain, infertility or frequent miscarriages etc. Moreover, long-term gluten consumption may lead to more severe symptoms. For example, weakening of the bones (osteoporosis) and iron deficiency anemia.

1.1 Regulations

According to the Codex Alimentarius (CODEX STAN 118/1979) food labeling is divided into to groups, "gluten-free" and "very low gluten" products where the gluten content is lower than 20 mg/kg and 100 mg/kg, respectively. For more information on the allergens' regulations, visit our website: <u>www.prognosis-biotech.com.</u>

1.2 Principle of the method

The presence of gluten in a sample is determined by the immunological detection of gliadin and other prolamins. Antibodies specific to gliadin are coated on the test line region (Test line) of the nitrocellulose membrane. During testing, antigens in the specimen react with the antibodies that are coated onto gold nanoparticles. The mixture migrates up the membrane to react with the antibodies immobilized on the membrane and generate a colored line in the test region T. The presence of the colored Test line indicates a positive result. In case of samples with a very high allergen concentration, the Test line fades or may not appear, giving us reduced or false negative result (hook effect phenomenon). For this purpose, a second line has been created (Hook line), whose intensity decreases as the amount of antigen increases and at very high concentrations it disappears either together with the Test line or before it. To serve as a procedural control, a colored line will always appear in the control region (Control line) if the test has been performed properly.

1.3. Kit Characteristics

See manual E1910/E1930 VN5



1.4 Method Protocol

Sample preparation

Solid Samples

- The sample must be collected according to established sampling techniques. Grind a representative sample (at least 5 g) to the particle size of fine instant coffee (50% passes through a 20 mesh screen).
- Weigh out a 1.0 g ground portion of the sample, add it into the prefilled sample tube and vortex it for 1 min. The ratio of sample to extraction solvent is 1:5 (w/v).
- Two (2) ml of the extract should be centrifuged at high speed for 10 min in reaction caps by using a microcentrifuge. Alternatively, let the sample settle down.
- Using a disposable pipette, transfer 1 drop from the supernatant to the empty extraction tube with the dropper tip.
- Dilute the sample **1:5** by adding 5 drops of the Matrix diluent (blue dropper tip) to the extraction tube with your supernatant.
- Close the extraction tube and shake well for a few seconds.

Liquid Samples

• Use 1.0 mL of the sample, add it into the prefilled sample tube and vortex it for 1 min. Follow the rest of the procedure exactly as described above for the solid samples. (You can skip using the microcentrifuge, unless your sample is a viscous liquid.)

Surfaces and swab sampling

- Mark out a swabbing area of approximately 10 x 10 cm.
- Moisten a swab by dipping into the extraction tube.
- Gather the sample with the swab by using a crosshatch technique. Move the swab horizontally, vertically, diagonally while rotating the tip. Repeat this starting from a different angle each time.
- After the sample collection, place the swab in the extraction tube, rotate the swab forcefully against the side of the tube for 1min. Best results are obtained when the sample is vigorously extracted in the solution. Remove the swab, squeezing the sides of the tube to extract as much liquid as possible. Shake vigorously for 1min on a vortex.
- Close the extraction tube. Add 1 drop into an empty extraction tube with the dropper tip. Dilute the sample 1:5 by adding 5 drops of the Matrix diluent (blue dropper tip).
- Close the extraction tube and shake well for a few seconds.

NOTE 1: The extracted sample should have a pH value of 6.2 - 7.5. If the pH is less than 6.2 as for example happens on the silage samples, the pH should be neutralized using NaOH or HCl.

NOTE 2: In case of cloudy, thick samples, that do not allow the mixture to develop, an extra dilution 1:1 with the Extraction Buffer is required before transferring 1 drop from the supernatant. In this case, multiply the final gluten ppm result x 2.



iii. Immunoassay Procedure and Interpretation of results (Total time: 5min)

• Add 3 drops in the circular window of the reaction device and allow the test to develop for 5 minutes.

Qualitative assessment

After 5 minutes, the test device can be visually read and interpreted as **1)Negative**, **2)Positive**, **3)High Positive** or **4) Invalid**, according to a Visual result interpretation index

Quantitative assessment

After 5 minutes, place the reaction device inside the plastic holder in order to be scanned. Use the appropriate software to quantify results as soon as possible and no later than 1 minute after the end of analysis. The software will use a Lot specific curve to calculate the results. Refer to the Reader's manual for a detailed description of the quantification procedure.



2 Immunoassay Specifications

2.1 General Specifications

- Visual LOD of the method is 3.0 ppm (3.0mg/kg) gluten or 1.5 ppm gliadin in food samples and CIP solutions.
- Visual LOD of the method is $1\mu g/100 \text{ cm}^2$ on working surfaces.

2.2 Assay Claims

- Samples showing negative results may contain Gluten below the limit of detection of the assay. This Lateral Flow kit does not claim that food is safe for consumption based upon a determination of gluten content. Matrix effects may also affect the result of the method.
- The recovery/cross reactivity of the method might be affected when analyzing processed food (e.g. heat treatment, dehydration, etc.), because proteins may be altered or fragmented.
- Food samples that have been heat treated may contain denatured proteins which may not be captured by the antibody. Recovery of these matrices might be reduced.
- A representative sample was used for the cross reactivity evaluation. Other samples may show a different result.
- The protein content and the protein composition may differ among various species of the same matrix. Therefore, different varieties may produce different results.
- LOD in CIP solutions refers to the final rinse water. The presence of cleaning agents and detergents may affect the result of the method.



3. Validation

3.1 Determination of the Limit of Detection LOD

The lowest detectable concentration of an analyte in a method is known as LOD. In this case, we check the concentration of a freshly prepared Gliadin from Wheat Extract, extracted and measured by **Allergen-Shield Gluten S** ELI-SA kit,A1048/A1096. The LOD is the level at which 95% of the replicates are characterized as positive. The results of 20 replicates of 6 dilutions with Gliadin Extract are shown at the table below.

Concentration (ppm)	Positive Replicates	Visual Interpretation of results
100	20/20	Strong Positive
50	20/20	Strong positive
25	20/20	Positive
10	20/20	Positive
5	20/20	Positive
3	20/20	Positive
1.5	3/20	Negative

Table 1. LOD of Gliadin proteins in liquid extract



3.2 Specificity & Cross-Reactivity

For the determination of cross-reactivity 10 replicates of gluten-free foods were tested. The samples were analyzed according to the experimental procedure described in Chapter 1.4. Four foods containing gluten also were tested (Table 3).

1 Almond 	No	Commodities	% Cross Reaction (N=10)	No	No Commodities	
2 Almond Flour <100	1	Almond	<lod< th=""><th>39</th><th>Peppercorn</th><th><lod< th=""></lod<></th></lod<>	39	Peppercorn	<lod< th=""></lod<>
3 Amaranth Flour -(LOD 41 Potato Flour/ Starch -(LOD 4 Arrowroot -(LOD 42 Pumphin Seed -(LOD 5 Barley 41 43 Quinoa Flour -(LOD 6 Black Bean -(LOD 44 Raw Shrimp -(LOD 7 Black Bean Flour -(LOD 44 Raw Shrimp -(LOD 8 Brown Rice -(LOD 47 Rye 85 10 Buckwheat -(LOD 47 Rye 85 10 Buckwheat Flour -(LOD 47 Rye 85 11 Buckwheat Flour -(LOD 48 Sesame Flour -(LOD 12 Cashew -(LOD 51 Soyg Bean -(LOD 13 Chestnut Flour -(LOD 52 Soya Flour -(LOD 16 Cocoa -(LOD 54 Sweet rice Flour -(LOD 17 Coconut -(LOD 57 Tapicoca <th>2</th> <th>Almond Flour</th> <th><lod< th=""><th>40</th><th>Pistachio</th><th><lod< th=""></lod<></th></lod<></th>	2	Almond Flour	<lod< th=""><th>40</th><th>Pistachio</th><th><lod< th=""></lod<></th></lod<>	40	Pistachio	<lod< th=""></lod<>
4Arrowroot<100	3	Amaranth Flour	<lod< th=""><th>41</th><th>Potato Flour/ Starch</th><th><lod< th=""></lod<></th></lod<>	41	Potato Flour/ Starch	<lod< th=""></lod<>
5Barley4143Quinoa Flour<1.0D	4	Arrowroot	<lod< th=""><th>42</th><th>Pumpkin Seed</th><th><lod< th=""></lod<></th></lod<>	42	Pumpkin Seed	<lod< th=""></lod<>
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14Chestnut Flour <lod< th="">52Soya Flour<lod< th="">15Chick Pea<lod< td="">53Spices<lod< td="">16Cocoa<lod< td="">54Sweet rice Flour<lod< td="">17Coconut<lod< td="">55Sunflower Seed<lod< td="">18Coconut Flour<lod< td="">56Tapicca<lod< td="">19Coffee<lod< td="">57Tapicca Flour/ starch<lod< td="">20Corn<lod< td="">58Tea<lod< td="">21Corn/Starch meal<lod< td="">59Triticale8822Dried Fruits<lod< td="">60Walnut<lod< td="">23Egg Powder<lod< td="">62Whey<lod< td="">25Flax seed Flour/ meal<lod< td="">63White bean Flour<lod< td="">26Garfava Flour<lod< td="">66Winite minot)<lod< td="">27Green Pea Flour<lod< td="">66Winite (merlot)<lod< td="">28Ground Beef<lod< td="">66Yantangum<lod< td="">30Ground Pork<lod< td="">69Lentils<lod< td="">31Ground Pork<lod< td="">70Lentils<lod< td="">33Hazelnut<lod< td="">72Lima Bean<lod< td="">34Hazelnut Flour<lod< td="">73Milk powder<lod< td="">35Kamut>7573Milk powder<lod< td="">36Oat Flour<lod< td="">74Millet<lod< td="">37Peanut<lod< td="">75Millet Flour<lod< td="">36Nat Fl</lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<>	13	Chestnut	<lod< th=""><th>51</th><th>Soy Bean</th><th><lod< th=""></lod<></th></lod<>	51	Soy Bean	<lod< th=""></lod<>
15Chick Pea <lod< th="">53Spices<lod< th="">16Cocoa<lod< td="">54Sweet rice Flour<lod< td="">17Coconut<lod< td="">55Sunflower Seed<lod< td="">18Coconut Flour<lod< td="">56Tapicca<lod< td="">19Coffee<lod< td="">57Tapica Flour/ starch<lod< td="">20Corn<lod< td="">58Tea<lod< td="">21Corn/Starch meal<lod< td="">60Walnut<lod< td="">23Fgg Powder<lod< td="">61Wheat10024Fava bean Flour<lod< td="">62Whey<lod< td="">25Grean Pea Flour<lod< td="">66White rice Flour<lod< td="">26Garfava Flour<lod< td="">66Wine (merlot)<lod< td="">29Ground Beef<lod< td="">66Xanthan gum<lod< td="">29Ground Pork<lod< td="">68Yellow pea Flour<lod< td="">30Ground Pork<lod< td="">68Yellow pea Flour<lod< td="">31Ground Turkey<lod< td="">68Yellow pea Flour<lod< td="">33Hazelnut<lod< td="">71Lima Bean<lod< td="">34Hazelnut<lod< td="">72Lima Bean Flour<lod< td="">36Oat Flour<lod< td="">74Millet Flour<lod< td="">36Peanut<lod< td="">75Millet Flour<lod< td="">37Peanut<lod< td="">76Oats<lod< td="">38Pecan<lod< td="">76Oats<lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<>	14	Chestnut Flour	<lod< th=""><th>52</th><th>Soya Flour</th><th><lod< th=""></lod<></th></lod<>	52	Soya Flour	<lod< th=""></lod<>
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22Dried Fruits <lod< th="">60Walnut<lod< th="">23Egg Powder<lod< td="">61Wheat10024Fava bean Flour<lod< td="">62Whey<lod< td="">25Flax seed Flour/ meal<lod< td="">63White bean Flour<lod< td="">26Garfava Flour<lod< td="">64White rice Flour<lod< td="">27Green Pea Flour<lod< td="">65Wine (merlot)<lod< td="">28Ground Beef<lod< td="">66Xanthan gum<lod< td="">29Ground Chicken<lod< td="">68Yellow pea Flour<lod< td="">30Ground Turkey<lod< td="">69Lentils<lod< td="">31Ground Turkey<lod< td="">70Lentil Flour<lod< td="">33Hazelnut<lod< td="">71Lima Bean<lod< td="">34Hazelnut Flour<lod< td="">72Lima Bean Flour<lod< td="">35Kamut>7573Milk powder<lod< td="">36Oat Flour<lod< td="">76Oats<lod< td="">38Pecan<lod< td="">76Oats<lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<>	21	Corn/Starch meal	<lod< th=""><th>59</th><th>Triticale</th><th>88</th></lod<>	59	Triticale	88
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26Garfava Flour <lod< th="">64White rice Flour<lod< th="">27Green Pea Flour<lod< td="">65Wine (merlot)<lod< td="">28Ground Beef<lod< td="">66Xanthan gum<lod< td="">29Ground Chicken<lod< td="">67Yeast (active dry)<lod< td="">30Ground Pork<lod< td="">68Yellow pea Flour<lod< td="">31Ground Turkey<lod< td="">69Lentils<lod< td="">32Guar gum<lod< td="">70Lentil Flour<lod< td="">33Hazelnut<lod< td="">71Lima Bean<lod< td="">34Hazelnut Flour<lod< td="">72Lima Bean Flour<lod< td="">35Kamut>7573Milk powder<lod< td="">36Oat Flour<lod< td="">74Millet Flour<lod< td="">37Peanut<lod< td="">76Oats<lod< td="">38Pecan<lod< td="">76Oats<lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<>	25	Flax seed Flour/ meal	<lod< th=""><th>63</th><th>White bean Flour</th><th><lod< th=""></lod<></th></lod<>	63	White bean Flour	<lod< th=""></lod<>
27Green Pea Flour <lod< th="">65Wine (merlot)<lod< th="">28Ground Beef<lod< td="">66Xanthan gum<lod< td="">29Ground Chicken<lod< td="">67Yeast (active dry)<lod< td="">30Ground Pork<lod< td="">68Yellow pea Flour<lod< td="">31Ground Turkey<lod< td="">69Lentils<lod< td="">32Guar gum<lod< td="">70Lentil Flour<lod< td="">33Hazelnut<lod< td="">71Lima Bean<lod< td="">34Hazelnut Flour<lod< td="">72Lima Bean Flour<lod< td="">36Oat Flour<lod< td="">74Millet pour<lod< td="">37Peanut<lod< td="">75Millet Flour<lod< td="">38Pecan<lod< td="">76Oats<lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<>	26	Garfava Flour	<lod< th=""><th>64</th><th>White rice Flour</th><th><lod< th=""></lod<></th></lod<>	64	White rice Flour	<lod< th=""></lod<>
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29Ground Chicken <lod< th="">67Yeast (active dry)<lod< th="">30Ground Pork<lod< td="">68Yellow pea Flour<lod< td="">31Ground Turkey<lod< td="">69Lentils<lod< td="">32Guar gum<lod< td="">70Lentil Flour<lod< td="">33Hazelnut<lod< td="">71Lima Bean<lod< td="">34Hazelnut Flour<lod< td="">72Lima Bean Flour<lod< td="">35Kamut>7573Milk powder<lod< td="">36Oat Flour<lod< td="">74Millet<lod< td="">37Peanut<lod< td="">76Oats<lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<>	28	Ground Beef	<lod< th=""><th>66</th><th>Xanthan gum</th><th><lod< th=""></lod<></th></lod<>	66	Xanthan gum	<lod< th=""></lod<>
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32Guar gum <lod< th="">70Lentil Flour<lod< th="">33Hazelnut<lod< td="">71Lima Bean<lod< td="">34Hazelnut Flour<lod< td="">72Lima Bean Flour<lod< td="">35Kamut>7573Milk powder<lod< td="">36Oat Flour<lod< td="">74Millet<lod< td="">37Peanut<lod< td="">75Millet Flour<lod< td="">38Pecan<lod< td="">76Oats<lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<>	31	Ground Turkey	<lod< th=""><th>69</th><th>Lentils</th><th><lod< th=""></lod<></th></lod<>	69	Lentils	<lod< th=""></lod<>
33 Hazelnut <lod< th=""> 71 Lima Bean <lod< th=""> 34 Hazelnut Flour <lod< td=""> 72 Lima Bean Flour <lod< td=""> 35 Kamut >75 73 Milk powder <lod< td=""> 36 Oat Flour <lod< td=""> 74 Millet <lod< td=""> 37 Peanut <lod< td=""> 75 Millet Flour <lod< td=""> 38 Pecan <lod< td=""> 76 Oats <lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<>	32	Guar gum	<lod< th=""><th>70</th><th>Lentil Flour</th><th><lod< th=""></lod<></th></lod<>	70	Lentil Flour	<lod< th=""></lod<>
34 Hazelnut Flour <lod< th=""> 72 Lima Bean Flour <lod< th=""> 35 Kamut >75 73 Milk powder <lod< td=""> 36 Oat Flour <lod< td=""> 74 Millet <lod< td=""> 37 Peanut <lod< td=""> 75 Millet Flour <lod< td=""> 38 Pecan <lod< td=""> 76 Oats <lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<>	33	Hazelnut	<lod< th=""><th>71</th><th colspan="2">71 Lima Bean</th></lod<>	71	71 Lima Bean	
35 Kamut >75 73 Milk powder <lod< th=""> 36 Oat Flour <lod< td=""> 74 Millet <lod< td=""> 37 Peanut <lod< td=""> 75 Millet Flour <lod< td=""> 38 Pecan <lod< td=""> 76 Oats <lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<></lod<>	34	Hazelnut Flour	<lod< th=""><th>72</th><th>Lima Bean Flour</th><th><lod< th=""></lod<></th></lod<>	72	Lima Bean Flour	<lod< th=""></lod<>
36Oat Flour <lod< th="">74Millet<lod< th="">37Peanut<lod< td="">75Millet Flour<lod< td="">38Pecan<lod< td="">76Oats<lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<>	35	Kamut	>75	73	Milk powder	<lod< th=""></lod<>
37 Peanut <lod< th=""> 75 Millet Flour <lod< th=""> 38 Pecan <lod< td=""> 76 Oats <lod< td=""></lod<></lod<></lod<></lod<>	36	Oat Flour	<lod< th=""><th>74</th><th>Millet</th><th><lod< th=""></lod<></th></lod<>	74	Millet	<lod< th=""></lod<>
38 Pecan <lod 76="" <lod<="" oats="" th=""><th>37</th><th>Peanut</th><th><lod< th=""><th>75</th><th>Millet Flour</th><th><lod< th=""></lod<></th></lod<></th></lod>	37	Peanut	<lod< th=""><th>75</th><th>Millet Flour</th><th><lod< th=""></lod<></th></lod<>	75	Millet Flour	<lod< th=""></lod<>
	38	Pecan	<lod< th=""><th>76</th><th>Oats</th><th><lod< th=""></lod<></th></lod<>	76	Oats	<lod< th=""></lod<>

Table 2. Cross-reactivities

All of the gluten-free matrices have no cross-reactivity at 100% with ProGnosis Biotech Gluten Free test.

Cross reactivities of the used antibodies have been determined for the pure food (e.g. corn flour). In a composed / processed food (e.g. maize bread) cross reactivities might be different.



Spike Protocol

In this study we want to check the capability of our product to detect gliadin in different matrices and to confirm that the LOD of our method is 3ppm, as we described earlier in 3.1. All the samples were spiked according to AOAC INTERNATIONAL Research Institute spiking protocol. More particularly, all spike experiments were carried out by spiking the individual pre-weighed test portion with a concentration adjusted solution to maintain the spiking volume at 100 μ L. The spike solution was prepared from Gliadin from wheat in ProGnosis Biotech Allergen-Shield Gluten S extraction buffer. Gliadin was weighed on an analytical balance with 0.001 g precision and dissolved in extraction buffer using a 50 mL volumetric flask. Gluten-free samples were spiked using the above prepared stock solution with a positive displacement Hamilton syringe and were left open in a fume hood for approximately 30 minutes to allow the solvent to evaporate prior extraction. For the surfaces, we chose plastic, stainless steel and synthetic (working bench) as the material. We cleaned a representative piece and we marked out 10 swabbing areas of approximately 10 x 10 cm. Each of these areas were spiked at 100 μ l with the stock solution and allowed to dry for 24 hours before sample collection. The resulting in-house spiked matrices and surfaces were extracted and analyzed according to the manual E1910/E1930 VN5 chapter 8.

i. Gluten Free edible materials (Food)

For the first Matrix Study, 3 representative gluten free matrices were spiked with the gliadin stock solution at 30ppm. Then, dilutions were made with Extraction buffer and, at each level, we used 10 replicates to check the efficiency of our product. Before we started, we confirmed that the samples did not contain gluten and that the spike level was right by using our ELISA kit Allergen-Shield Gluten S, A1048/A1096.

Matrix	Number of Reaction Device (N=10)							Average				
WIGUIA	Dilucion	1	2	3	4	5	6	7	8	9	10	Average
	30ppm	+	+	+	+	+	+	+	+	+	+	10/10
Break-	1/5 (6ppm)	+	+	+	+	+	+	+	+	+	+	10/10
fast cereal	1/10 (3ppm)	+	+	+	+	+	+	+	+	+	+	10/10
	1/20 (1.5ppm)	-	-	-	+	-	-	-	+	-	-	02/10
	30ppm	+	+	+	+	+	+	+	+	+	+	10/10
Cake mix	1/5 (6ppm)	+	+	+	+	+	+	+	+	+	+	10/10
	1/10 (3ppm)	+	+	+	+	+	+	+	+	+	+	10/10
	1/20 (1.5ppm)	+	-	-	-	-	-	+	-	-	-	02/10
	30ppm	+	+	+	+	+	+	+	+	+	+	10/10
Cooked biscuit	1/5 (6ppm)	+	+	+	+	+	+	+	+	+	+	10/10
	1/10 (3ppm)	+	+	+	+	+	+	+	+	+	+	10/10
	1/20 (1.5ppm)	-	+	-	-	-	-	-	-	-	-	01/10

Table 3. Matrix study of Gluten Free Test on food



ii. Clean in Place Rinses (CIP SOLUTIONS)

For this study, 2 representative CIP solutions and Deionized water were spiked with the gliadin stock solution at 30ppm. Then, dilutions were made with Extraction buffer and, at each level, we used 10 replicates to check the efficiency of our product. Before we started, we confirmed that the CIPs did not contain gluten and that the spike level was right by using our ELISA kit Allergen-Shield Gluten S, A1048/A1096.

Matrix	Dilution	Number of Reaction Device (N=10)										
Watrix	Dilution	1	2	3	4	5	6	7	8	9	10	Aveluge
	30ppm	+	+	+	+	+	+	+	+	+	+	10/10
Aciplus	1/5 (6ppm)	+	+	+	+	+	+	+	+	+	+	10/10
foam 2,5%	1/10 (3ppm)	+	+	+	+	+	+	+	+	+	+	10/10
	1/20 (1.5ppm)	+	-	-	-	-	-	-	-	-	+	02/10
Divosan VT5 1,8%	30ppm	+	+	+	+	+	+	+	+	+	+	10/10
	1/5 (6ppm)	+	+	+	+	+	+	+	+	+	+	10/10
	1/10 (3ppm)	+	+	+	+	+	+	+	+	+	+	10/10
	1/20 (1.5ppm)	-	-	-	-	-	-	+	-	-	-	01/10
Deion- ized water	30ppm	+	+	+	+	+	+	+	+	+	+	10/10
	1/5 (6ppm)	+	+	+	+	+	+	+	+	+	+	10/10
	1/10 (3ppm)	+	+	+	+	+	+	+	+	+	+	10/10
	1/20 (1.5ppm)	-	-	-	-	-	+	-	-	-	-	01/10

Table 4. Matrix study of Gluten Free Test on CIP solutions



iii. Surfaces-Swab Technique

For the surfaces, we chose plastic, stainless steel and synthetic (working bench) as the material. We cleaned a representative piece and we marked out 10 swabbing areas of approximately 10 x 10 cm. Each of these areas were spiked at 100µl with the stock solution and allowed to dry for 24 hours before sample collection. For each surface, the following replicate test portions were prepared: 10 at a gliadin level of $5\mu g/100 \text{ cm}^2$, 10 at a level of $2\mu g/100 \text{ cm}^2$, 10 at a level of $1\mu g/100 \text{ cm}^2$, and 10 at a level of $0.5\mu g/100 \text{ cm}^2$. The result of this study is that the LOD of our product, for environmental swabs, is $1\mu g/100 \text{ cm}^2$.

Surface	Snike level	Number of Reaction Device (N=10)											
Surface	Spike level	1	2	3	4	5	6	7	8	9	10	Aveluge	
	5μg/100 cm ²	+	+	+	+	+	+	+	+	+	+	10/10	
Chaimlana	2μg/100 cm ²	+	+	+	+	+	+	+	+	+	+	10/10	
steel	1μg/100 cm ²	+	+	+	+	+	+	+	+	+	+	10/10	
0	0,5μg/100 cm ²	-	+	-	-	-	-	-	-	-	+	02/10	
Plastic 1.	5μg/100 cm ²	+	+	+	+	+	+	+	+	+	+	10/10	
	2μg/100 cm ²	+	+	+	+	+	+	+	+	+	+	10/10	
	1μg/100 cm ²	+	+	+	+	+	+	+	+	+	+	10/10	
	0,5μg/100 cm²	-	-	+	-	-	-	+	-	+	-	03/10	
	5µg/100 cm²	+	+	+	+	+	+	+	+	+	+	10/10	
Synthetic	2μg/100 cm ²	+	+	+	+	+	+	+	+	+	+	10/10	
	1μg/100 cm ²	+	+	+	+	+	+	+	+	+	+	10/10	
	0,5μg/100 cm ²	-	-	-	-	-	+	-	-	-	-	01/10	

Table 5. Matrix study of Gluten Free Test on surfaces



3.4 Performance Evaluation with Reference Materials

To evaluate the efficiency of Gluten Free Test, we chose a wide range of reference materials that had been treated from FAPAS, an accredited proficiency testing provider. All samples were accompanied by certificates that confirm their assigned value. For each sample, 10 replicates were performed.

Reference material	Assigned value (mg/kg) according to R-Biopharm Ridascreen Gliadin	Range for z ≤2	Result (N=10)	Average
FAPAS Cake Mix	34,7	17,3 - 52,0	Positive	10/10
FAPAS Cake Mix	34,3	17,1 - 51,4	Positive	10/10
FAPAS Cooked Bis-	76,0	38,0 - 114,0	Positive	10/10
FAPAS Infant Soya Formula T27240AQC	NOT DETECTED	ED - <lod< td=""><td>10/10</td></lod<>		10/10
FAPAS Infant Soya Formula T27240BQC	37,1	18,5 - 55,6	Positive	10/10
FAPAS Infant Soya Formula T27264AQC	NOT DETECTED	-	<lod< td=""><td>10/10</td></lod<>	10/10
FAPAS Infant Soya Formula T27264BQC	24,8	12,4 - 37,2	Positive	10/10
FAPAS Oat Based Foodstuff T27228AQC	NOT DETECTED	-	<lod< td=""><td>10/10</td></lod<>	10/10
FAPAS Oat Based Foodstuff T27228BQC	45,0	22,5 - 67,5	Positive	10/10
FAPAS Oat Based Foodstuff T27252AQC	16,6	8,3 - 24,9	Positive	10/10
FAPAS Oat Based Foodstuff T27252BQC	NOT DETECTED	-	<lod< td=""><td>10/10</td></lod<>	10/10

	Table 6. Evaluation	of Gluten Free	Test with samples	prepared by FAPAS
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www.prognosis-biotech.com E: info@prognosis-biotech.com

T: +30 2410 623922 Farsalon 153 | 41335 Larissa, Greece