GLUTEN HYDROLYZED ELISA KIT (anticorps R5)

Référence LIBIOS : GLU-HYD-R5-48P

Direct immunoenzymatic assay for quantitative analysis of hydrolyzed gluten in food samples

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KIT COMPOSITION

Reactivo Reagent	**Unidad**	**Volumen**
Six breakable strips (6x8 wells) activated for capturing prolamins	6 tiras	
European Gliadin Standard. Original standard point 400 ppm (100x concentrated)	1	200 µL
Anti-prolamins Mab-peroxidase conjugate (ready to use)	1	8 mL
Extraction buffer ready to use	1	110 mL
Washing solution 25x concentrated	1	65 mL
Diluent DE29	1	65 mL
Substrate (TMB)	1	15 mL
Stop Solution (H_2SO_4 0,5M) *(*)*	1	15 mL

(*) Caution: This solution is acid. Avoid contact with eyes and skin. In case of skin contact, wash immediately with copious amounts of water. A mild soap should be used. In case of eye contact, flush generously with water for at least 15 minutes. Seek urgent medical attention if the irritation persists or if it is severe.

OTHER MATERIALS AND REAGENTS NEEDED NOT PROVIDED WITH THE KIT:

Ethanol
Distilled water

I. TECHNICAL BASIS

GLUTEN HYDROLYZED is a direct enzyme immunoassay. It can identify and quantify hydrolyzed prolamins (gliadina, hordein and secalins).

Prolamins extracted from the sample are chemically fixed to the polystyrene plate wells. After a washing step, the peroxidase-conjugated R5 Mab is added, which will bind to the fixed prolamins. After the addition of the substrate, a colorimetric reaction occurs. This reaction is directly proportional to the amount of gluten in the well.

With GLUTEN HYDROLYZED is possible to quantify the amount of hydrolyzed gluten of a sample with a detection limit of **0,25 ppm.**

II. PRECAUTIONS AND WARNINGS FOR USERS

1. Read the use instructions carefully.
2. Bring all reagents to room temperature prior to use.
3. Do not mix instructions or reagents from different kits.
4. Avoid any contamination of the reagents of the Kit.
5. Do not use components after expiration dates and do not mix components from different lots.
6. There should be no eating, drinking, or smoking where specimens or Kit reagents are being handle.
7. Do not pipette by mouth.
8. Use a new tip for every new sample.
9. Include the controls and the standard curve every time the assay is run.
10. **IMPORTANT:** TMB Substrate must be handled with care, as it is very sensitive to light and contamination: pipette a sufficient amount for the assay from the substrate storage bottle into a separate container prior to colour development step. Do not return the non used reagent to the storage bottle.
11. Stop solution is a strong acid. Handle with care

III. STORAGE OF THE KIT COMPONENTS

- ✓ All components must be stored between +2ºC and +8ºC. DO NOT FREEZE ANY OF THE KIT COMPONENTS.
- ✓ Avoid exposure of the kit and the components to direct sunlight at any time, as some reagents are light sensitive.

IV. INFORMATION ABOUT THE WASHING STEPS

The washing steps could be done using an automatic washing machine or a squeeze bottle or a multichanel pipetting device suitable for dispensing 300 µL on each well. After the incubation periods, the washing steps must be done following these instructions:

- Throw out the content of the plate by a brusque turn over of the plate to avoid the possible mixture of the content from one well to another.
- Dispense a volume of 300 µL of washing solution on every well.
- Shake the plate gently, avoiding the contamination between wells.
- Turn over the plate brusquely to empty the wells.
- Repeat the process as many times as indicates the kit's instructions.
- Prior to emptying the content of the last washing step, verify that the next reagent to be added is ready to use. Do not let the plate dry longer than strictly necessary.
- After emptying the contents of the last washing step, tap the plate turned over on absorbent filter paper to remove the remaining washing solution.

V. PREPARATION OF REAGENTS

Conjugate
It is supplied ready to use. Do not dilute. Add 100 µL directly to the well.

Gliadin European standard (400 ppm). Original standard point (100X concentrated):

The original standard point must be diluted 1/100 in diluent (put 10 µl of the original point into 990 µl of diluent). This dilution corresponds to 4 ppm of gluten. Add 100 µl of diluent in five wells. Add 100 µl of the diluted original point in the first well. Then, transfer 100 µl of this first well to the second well. Repeat the operation until the last well and remove 100 µl of this last well. The first well (2 ppm) will be the positive control and the standard curve should be performed with the rest of the wells (from 1 ppm to 0.125 ppm). Always add 100µL of diluent to a separate well as a Negative Control.

It is recommended changing the tip with every dilution and mix well before transferring the 100µL to the following well (for mixing, take the 100µL volume with the pipette and throw it into the well 2 or 3 times).

Very important: the curve must be prepared at the moment, every time the assay is going to be performed

Washing solution:
Dilute one part of the concentrate washing solution provided in the kit with 24 parts of distilled or deionized water. When ready, this solution remains stable between +2ºC and +8°C.

VI. SAMPLES PREPARATION

IMPORTANT: A special extraction procedure should be used for samples containing chocolate. Please, contact with your distributor.

A representative piece of product should be taken. This must be ground to acquire a fine consistency to provide a homogeneous mixture.

Solid Samples

1. Weigh 0,25 g of sample previously homogenized and put it in a polypropylene tube of 10 mL.
2. Add 2,5 mL of extraction buffer and Vortex (10-15 seconds).
3. Incubate for 30 minutes at room temperature.
4. Add 7,5 mL of 80% ethanol and incubate 10 minutes at room temperature with agitation.
5. Centrifuge 10 min at 10.000 rpm.
6. Transfer the supernatant to clean polypropylene tubes and then analyse by ELISA.

Liquid samples

1. Measure a volume of 5 mL 60% ethanol and put it in a 10 mL polypropylene tube.
2. Add 125 µL of the sample and Vortex (10-15 seconds).
3. Incubate 5 minutes at room temperature.
4. This is the sample ready to be analyzed in the ELISA.

Before being added to the ELISA plate wells, the samples should be diluted as follows:

To detect absence or presence of hydrolyzed gluten.

- *For samples with estimated hydrolyzed gluten content lower than 0,25 ppm add 50 µL of the diluent in the well and then 50 µL of the sample.*

To quantify hydrolyzed gluten

- *Samples with estimated hydrolyzed gluten content lower than 20 ppm: make dilution 1/10, using the supplied diluent.*
- *Samples with hydrolyzed gluten content between 20-50 ppm: make dilutions 1/20, using the supplied diluent.*
- *Samples with gluten content between 50-100 ppm: make dilutions 1/40, using the supplied diluent.*

. *Samples with hydrolyzed gluten content higher than 100 ppm: make dilutions 1/100, using the supplied diluent.*

Dilution procedure:

1/10 = 900 µL of diluent + 100 µL of sample.
1/20 = 950 µL of diluent + 50 µL of sample.
1/40 = 975 µL of diluent + 25 µL of simple.
1/100 = 990 µL of diluent + 10 µL of sample.

VII. TEST PROCEDURE

All reagents must be allowed to come to room temperature before use.

Addition of samples and controls. Both samples and standards dilution must be prepared just before performing the assay

VERY IMPORTANT. In order to keep the samples and the curve in the same conditions, make the dilutions of samples and the dilution of the original point immediately one after the other. Never dilute the samples, put them into the wells and then dilute the original point to perform the standard curve.

To determine absence or presence of hydrolyzed gluten.

1. Add 50 µL of diluent in the wells where samples will be dispensed.
2. Add 50 µL of the extracted samples (without dilution). It is recommended to include a positive control and 100 µl of diluent as negative control.

To quantify hydrolyzed gluten.

1. Add 100 µL of the samples (diluted according to instructions above) by duplicate. Then perform the standard curve following the instructions of point V.
2. Seal the plate and **incubate for 30 minutes at room temperature (22-25 º C).**
3. Wash 6 times as described above.
4. Add 100 µL of specific conjugate to the wells Seal the plate and **incubate 30 minutes at room temperature (22-25°C)**.
5. Wash 6 times as described above.
6. Add 100 µL of TMB substrate to the wells. Keep the plate for **15 min at room temperature.** The use of a multichannel pipette is recommended.
7. Add 100 µL of stop solution to each well following the substrate's order of addition. A positive reaction will change the colour from blue to yellow.
8. Read the OD of each well at 450 nm within 5 min alter the addiction of stop solution.

VIII. READING AND INTERPRETATION OF THE RESULTS

Read the OD of each well at 450 nm.

To determine absence or presence of hydrolyzed gluten

If colour appears, it indicates the presence of gluten in the sample. High colour intensity does not mean a high amount of gluten in the sample.

To quantify hydrolyzed gluten

Validation of the test:

The test should be considered as valid when the OD of 2 ppm point is higher than the OD of the 1ppm point; and de OD of the negative control is lower than the OD of 0,125ppm point.

Results interpretation:

The standards of the kits already take into consideration the dilution factor performed during the extraction step indicated in this package insert. So, additional multiplied factor should not be used.

The ppm of the sample are calculated from the following formula:

ppm= C x D where:

C is the ppm concentration obtained from the curve

D is the dilution applied to the extracted sample

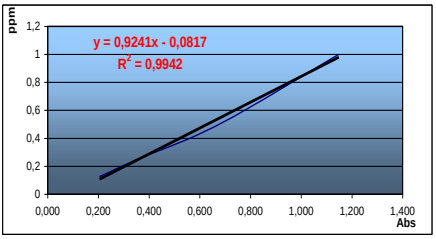
◇ The hydrolyzed gluten content of the sample is quantified as follows:

- Plot the calibration curve (see example) using the absorbances of the four points of standard curve.
- Add trend line and add a lineal equation (see example).

- Determine the concentration of hydrolyzed gluten sample by interpolating the absorbance value in the curve (see example). To achieve this, use the appropriate software. For example:

  - ✓ Software: Excel (Microsoft)
  - ✓ Select the standards concentrations and their correspondent OD values. Then click on "Graphic assistant"
  - ✓ Select type of graphic: "XY (dispersion)"
  - ✓ Click on graphic windows and select "Add tendency line"
  - ✓ In label "type", click on "LINEAL"
  - ✓ In label "Options", click on "Present formula in the graphic"
  - ✓ In this formula, replace the unknown factor "x" by the sample OD value to obtain the ppm of HYDROLYZED GLUTEN.
  - ✓ The final content of gluten of the sample will be obtained by multiplying the concentration from the standard curve by the applied dilution

Abs 450	ppm
2,326	Control pos
1,148	1
0,676	0,5
0,354	0,25
0,205	0,125
0,058	Control neg



Example:

	Abs 450nm	C	D	ppm
Sample A	0,85	0,82	2	1,6
Sample B	1,05	1,04	20	20,8
Sample C	0,96	0,94	100	94,1

ADDITIONAL INFORMATION

- ➢ To assure that the calculations are correct, the absorbance values of samples should be in the range of curve (0,125 - 1 ppm of gluten). It may be necessary to dilute the sample to achieve these results. If so, it is important to apply the dilution factor in the calculation of results.
- ➢ Just in case the OD value of the sample is slightly higher than the OD of the 1 ppm point (up to 15%), it can be interpolated into de curve, including the positive control (2ppm) into de curve.
- ➢ Low values of the calibration curve are often caused by incubation temperature or because too much time has passed between the construction of the curve and the performance of the assay. Make sure you are at these limits (22-25°C).Perform the standard curve making serial dilution as indicated in the instructions.

IX. OVERVIEW OF PROCEDURE

1. Reagents must be equilibrated at room temperature (22-25ºC) before start.
2. Make the samples dilution and the original point dilution
3. Add 100 µL of **samples** (prepared and diluted as described in point VI) in duplicated wells.
4. Perform the curve in the wells and add 100 µl of diluent as **negative control**. Seal the plate.
5. Incubate at room temperature (22-25ºC) for **30 minutes**.
6. Wash wells 6 times with diluted wash solution (1x) to remove not bound reagents.
7. Add 100 µL of **Conjugated-**Peroxidase to every well. Seal the plate.
8. Incubate at room temperature (22-25ºC) for **30 minutes**.
9. Wash wells 6 times with diluted wash solution (1x).
10. Add 100 µL of substrate (TMB) to every well.
11. Incubate **15 minutes** at room temperature.
12. Add 100 µL of stop solution to every well.
13. Read at 450 nm.

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