Glutelin Assay Kit

ARG83403 Glutelin Assay Kit can be used to measure Glutelin in tissue extracts and powder.

Catalog number: ARG83403

Package: 96 wells

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INTRODUCTION

Glutelins are a class of prolamin proteins found in the endosperm of certain seeds of the grass family. They constitute a major component of the protein composite collectively referred to as gluten. Glutenin is the most common glutelin, as it is found in wheat and is responsible for some of the refined baking properties in bread wheat. The glutelins of barley and rye have also been identified. Glutelins are the primary protein form of energy storage in the endosperm of rice grains.

PRINCIPLE OF THE ASSAY

The color is measured at a wavelength of 595nm ±2nm. The concentration of Glutelin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	-20°C
Assay Buffer A	2 X 30 ml (ready to use)	4°C
Assay Buffer B	2 X 30 ml (ready to use)	4°C
Assay Buffer C	2 X 30 ml (ready to use)	4°C
Assay Buffer D	2 X 30 ml (ready to use)	4°C
Reagent Dye	20 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 595 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Standard store at-20°C, all other component store at 4°C.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue- Weigh out 0.05 g tissue, homogenize with 0.5 ml Assay Buffer I on ice, transfer it to centrifuge tube and mix on a lab rotator for 30 minutes; centrifuged at 10000g 4 °C for 10 minutes, discard the supernatant; then add 0.5 ml Assay Buffer II into the tube, mix on a lab rotator for 30 minutes; centrifuged at 10000g 4 °C for 10 minutes, discard the supernatant; then add 0.5 ml Assay Buffer III into the tube, mix on a lab rotator for 30 minutes; centrifuged at 10000g 4 °C for 10 minutes, discard the supernatant; then add 0.5 ml Assay Buffer IV into the tube, mix on a lab rotator for 30 minutes,

centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Powder- Weigh out 0.05 g powder, add 0.5 ml Assay Buffer I to dissolve, mix on a lab rotator for 30 minutes; centrifuged at 10000g 4 °C for 10 minutes, discard the supernatant; then add 0.5 ml Assay Buffer II into the tube, mix on a lab rotator for 30 minutes; centrifuged at 10000g 4 °C for 10 minutes, discard the supernatant; then add 0.5 ml Assay Buffer III into the tube, mix on a lab rotator for 30 minutes; centrifuged at 10000g 4 °C for 10 minutes, discard the supernatant; then add 0.5 ml Assay Buffer IV into the tube, mix on a lab rotator for 30 minutes, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

REAGENT PREPARATION

- Standard: Add 1 ml of distilled water to yield 2 mg/mL standard. Perform 2-fold serial dilution of the top standards to make the standard curve.
- Sample: If the measuring absorbance of samples is higher than the standard, dilute the samples with Distilled water before assay and assay again. For the calculation of the activity this dilution factor has to be taken into account.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **10 µl** of **Sample** into Sample wells.
2. Standard wells: Add **10 µl** of **Standard** into Standard wells.
3. Add **200 µl Reagent Dye** to each wells.
4. Mix well. Incubate at **RT** for **2 min**. Read the OD at **595nm**

Reagent	Sample	Standard	Blank
Sample	10 µl	-	-
Standard	-	10 µl	-
Distilled water	-		10 µl
Reagent Dye	200 µl	200 µl	200 µl
Mix well. Incubate at RT for 2 min. Read the OD at 595nm			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

2. Calculation:

A. Definition:

$C_{Standard}$: the standard concentration, 2 mg/ml;

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 10 µl = 0.01 ml;

$V_{standard}$: the volume of standard sample, 10 µl = 0.01 ml;

V_{assay}: the volume of Assay Buffer III, 500 µl = 0.5 ml.

B. Formula:

a). According to the weight concentration of sample

Glutelin (mg/g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample)} - OD_{Sample})] / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{assay)}]$

$=4X (OD_{Sample} - OD_{Sample}) / [(OD_{Standard} - OD_{Blank}) X W]$

3. Detection range:

The detection range is from 0.02 mg/ml- 2 mg/ml.

4. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.