Glutelin

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1200

(Version 1.4A)

Detection and Quantification of GlutelinContent in Tissue extracts, PowderSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Glutelin is the primary storage protein in cereal grains, crucial for determining dough elasticity and food processing quality. Detection of glutelin content is of great significance for evaluating the nutritional value of grains, food processing suitability, and gluten-free diet management.

GlutelinColorimetric Microplate Assay Kit is a sensitive assay for determining Glutelin content in plant samples. The color intensity, measured at 595 nm, is proportionate toGlutelin content in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 2	4 °C
Assay Buffer II	30 ml x 2	4 °C
Assay Buffer III	30 ml x 2	4 °C
Assay Buffer IV	30 ml x 2	4 °C
Dye Reagent	20 mlx 1	4 °C, keep in dark
Standard	Powder x 1	-20 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 595 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
9. Lab rotator

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate2 mg/ml of standardtop solution, store at 4 °C for 2-3 weeksor -20°C for 6 months after reconstitution.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be2/1/0.5/0.25/0.125/0.0625/0.0312mg/ml.

V. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.05 g tissue, homogenize with 0.5mlAssay BufferI on ice, transfer it to centrifuge tube and mix on a lab rotatorfor 30 minutes; centrifuged at 10000g 4°C for 10minutes, discard the supernatant;then add 0.5mlAssay Buffer II into the tube, mix on a lab rotatorfor 30 minutes; centrifuged at 10000g 4°C for 10minutes, discard the supernatant;then add 0.5mlAssay Buffer III into the tube, mix on a lab rotatorfor 30 minutes; centrifuged at 10000g 4°C for 10minutes, discard the supernatant;then add 0.5mlAssay Buffer IV into the tube, mix on a lab rotatorfor 30 minutes,centrifuged at 10000g 4°C for 10minutes,take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For powder samples

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

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Dye Reagent	190µl	190µl	190µl
Mix, wait for 2 minutes, measured at 595 nm and recordthe absorbance.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mg/ml)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu g/g)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mg/ml = μg/μl

$V_{Standard}$: the volume of standard in assay procedure, μl

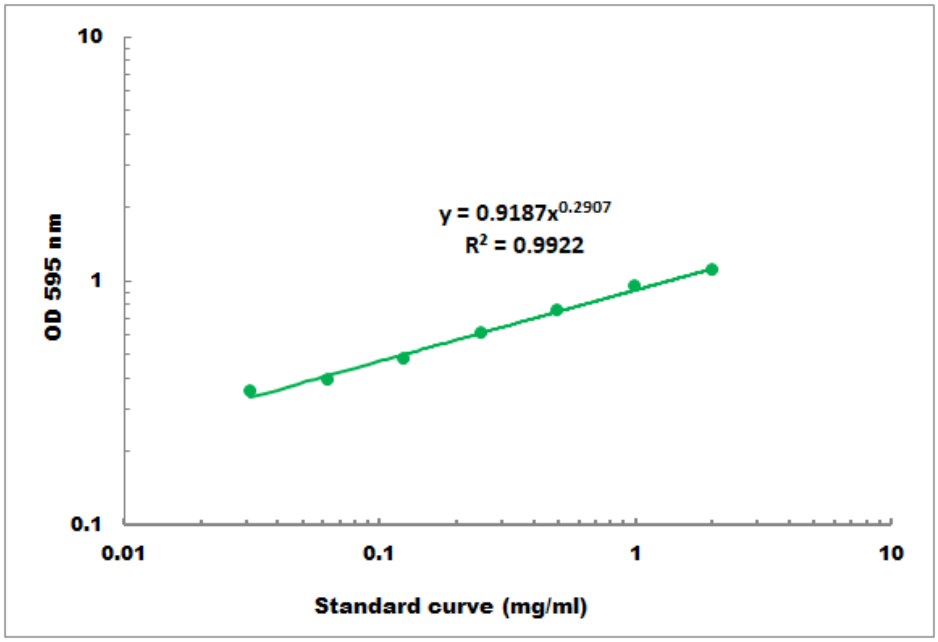
V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

W: the weight of sample, g

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.02mg/ml - 2mg/ml