

Glutelin Microplate Assay Kit

Cat #: orb707361 (manual)

Detection and Quantification of Glutelin Content in Tissue extracts, Powder Samples.

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INTRODUCTION

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

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 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Standard: add 1 ml distilled water to dissolve before use, the concentration will be 2 mg/ml.

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

SAMPLE PREPARATION

1. For tissue samples

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.

2. For powder samples

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.

ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	10 μ l	--	--
Standard	--	10 μ l	--
Distilled water	--	--	10 μ l
Dye Reagent	200 μ l	200 μ l	200 μ l

Mix, wait for 2 minutes, measured at 595 nm and record the absorbance.

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) 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.

CALCULATION

1. According to the weight of sample

$$\begin{aligned}\text{Glutelin (mg/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / ((\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times W / V_{\text{Assay}})) \\ &= 4 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

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

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

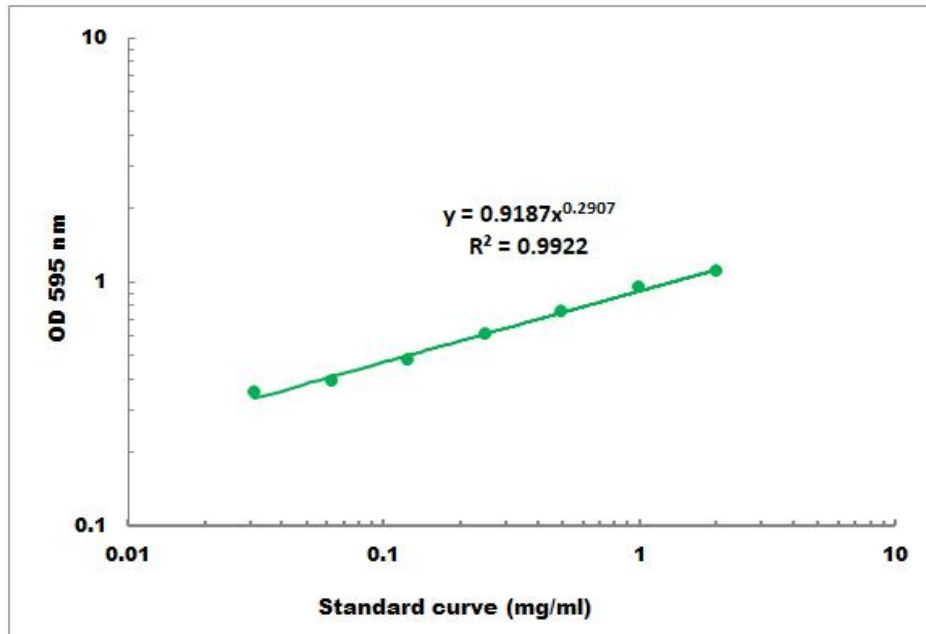
V_{Sample} : the volume of sample, 10 μl = 0.01 ml;

W: the weight of sample, g;

V_{Assay} : the volume of Assay Buffer III, 0.5 ml.

TYPICAL DATA

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



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