



Glutelin

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1200

(Version 1.3A)

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

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

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	3
IV. SAMPLE PREPARATION.....	4
V. ASSAY PROCEDURE.....	5
VI. CALCULATION.....	6
VII. TYPICAL DATA.....	7
VIII. TECHNICAL SUPPORT.....	7
IX. NOTES.....	7

I. INTRODUCTION

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 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.

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. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.05 g tissue, homogenize with 0.5ml 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 0.5ml 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 0.5ml 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 0.5ml 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 0.5ml 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 0.5ml 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 0.5ml 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 0.5ml 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.

V. 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.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

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

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

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

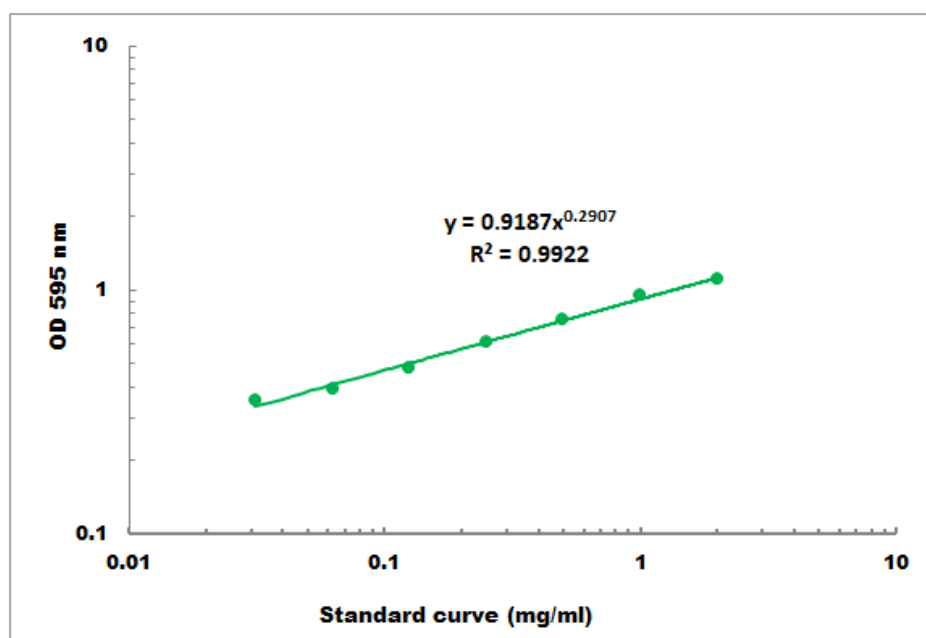
V_{Sample} : the volume of sample, 10 μl = 0.01 ml;

W : the weight of sample, g;

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

VII. 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES