



Hordein

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1199

(Version 1.4A)

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

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

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I. INTRODUCTION

Hordein is the main storage protein in barley grains, and its content and composition directly affect the processing quality and nutritional properties of barley. Detection of Hordein is of great significance for evaluating the brewing suitability, food allergenicity, and variety selection of barley.

HordeinColorimetric Microplate Assay Kit is a sensitive assay for determining Hordein content in plant samples. The color intensity, measured at 595 nm, is proportionate to Hordein 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
Dye Reagent	20 ml x 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. Dissolve in 1 ml distilled water to generate 2 mg/ml of standard top solution, store at 4 °C for 2-3 weeks or -20°C for 6 months after reconstitution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 2/1/0.5/0.25/0.125/0.0625/0.0312 mg/ml.

V. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.05 g tissue, homogenize with 0.5ml Assay Buffer I on ice, transfer it to a centrifuge tube and mix on a lab rotator for 30 minutes; centrifuge 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; centrifuge 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, centrifuge 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; centrifuge 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; centrifuge 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, centrifuge at 10000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. 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	190 μ l	190 μ l	190 μ l
Mix, wait for 2 minutes, measured at 595 nm and record the 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. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

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

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

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

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, $\text{mg/ml} = \mu\text{g}/\mu\text{l}$

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

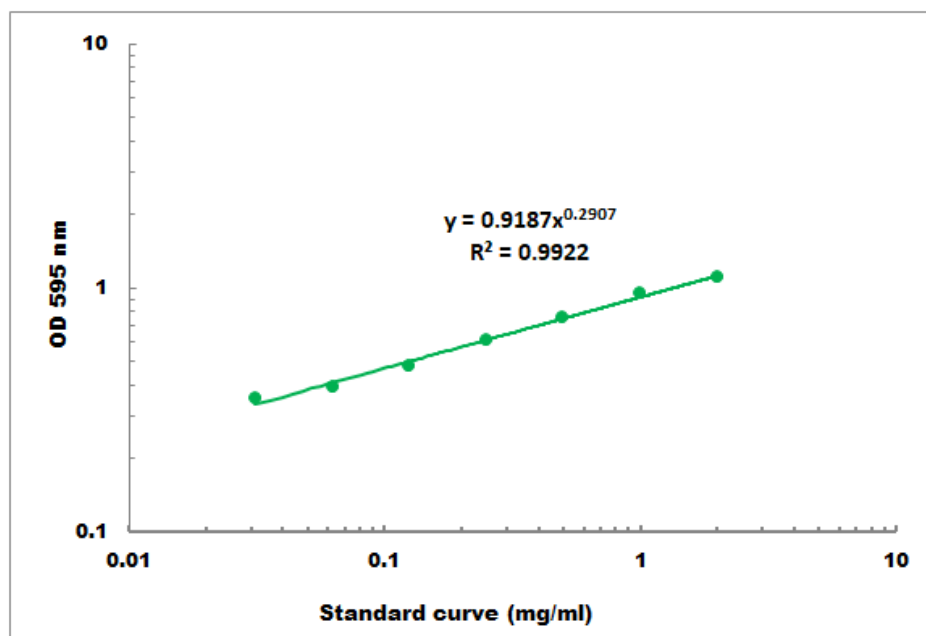
V_{Sample} : the volume of sample in assay procedure, μl

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