Hordein

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1199

(Version 1.3A)

Detection and Quantification of HordeinContent in Tissue extracts, PowderSamples.

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

I. INTRODUCTION

HordeinColorimetric Microplate Assay Kit is a sensitive assay for determining Hordein content in plant samples. The color intensity, measured at 595 nm, is proportionate toHordein 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 mlx 1	4 °C
Standard	Powder x 1	-20 °C
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Note:

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

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, 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, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Dye Reagent	200µl	200µl	200µl
Mix, wait for 2 minutes, measured at 595 nm and recordthe 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

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

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

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

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

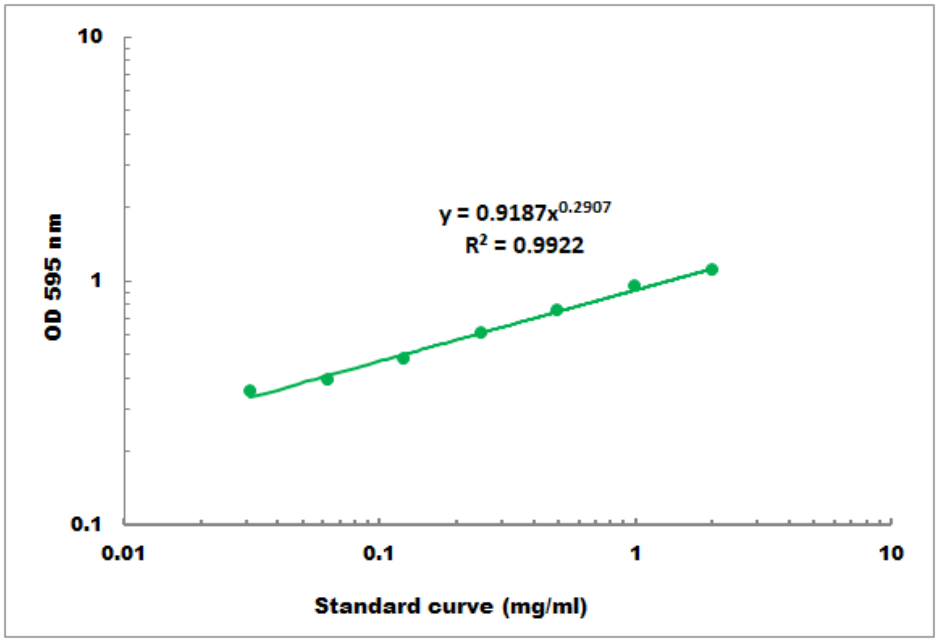
V_{Sample}: the volume of sample,10 µl = 0.01 ml;

W: the weight of sample, g;

V_{Assay}: the volume of Assay Buffer III, 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 towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES