Hordein Microplate Assay Kit

Cat #: orb707360 (manual)

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

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

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

INTRODUCTION

Hordein 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.

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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
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, 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, 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 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Dye Reagent	200 µl	200 µl	200 µl
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

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

$$= 4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: 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.

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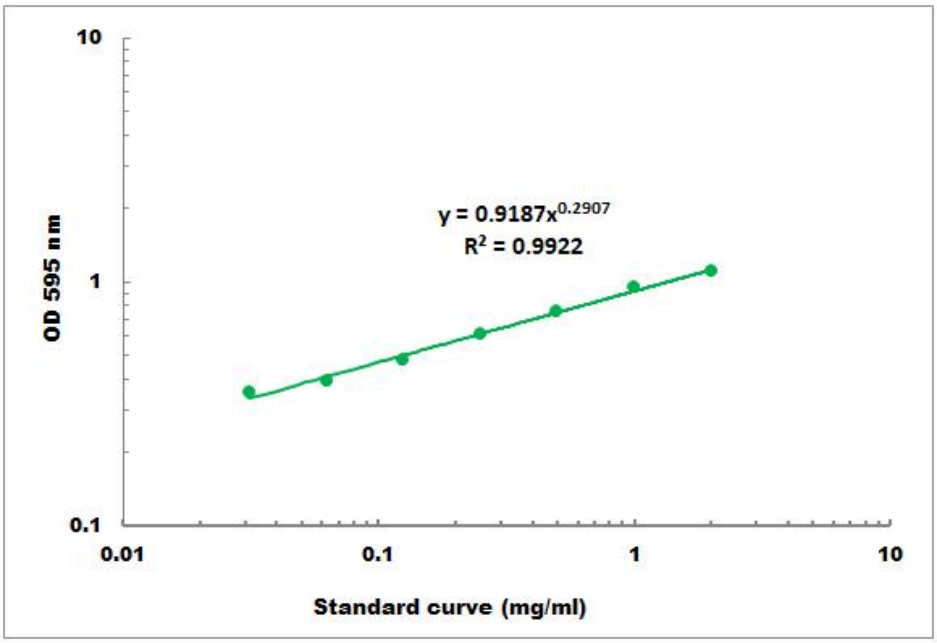
TYPICAL DATA

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

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