Hordein Assay Kit

ARG83402 Hordein Assay Kit can be used to measure Hordein in tissue extracts and powder.

Catalog number: ARG83402

Package: 96 wells

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INTRODUCTION

Hordein is a prolamin glycoprotein, present in barley and some other cereals, together with gliadin and other glycoproteins (such as glutelins) coming under the general name of gluten. Hordeins are found in the endosperm where one of their functions is to act as a storage unit.

PRINCIPLE OF THE ASSAY

The Hordein Assay Kit can measure Hordein in plant samples. The increase in absorbance at 595 nm is directly proportional to the Hordein content.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard (2mg)	1 vial (lyophilized)	-20°C
Assay Buffer I	2 X 30 ml (ready to use)	4°C
Assay Buffer II	2 X 30 ml (ready to use)	4°C
Assay Buffer III	2 X 30 ml (ready to use)	4°C
Reagent Dye	20 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 595 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Standard store at -20°C, all other component store at 4°C.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue - 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

Powder - 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.

REAGENT PREPARATION

- **Standard:** Add **1 ml** of **distilled water** to yield **2 mg/ml** standard. Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Sample**: If the measuring absorbance of samples is higher than the standard, dilute the samples with **Distilled water** before assay and assay again. For the calculation of the activity this dilution factor has to be taken into account.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **10 µl Sample** into Sample wells.
2. Standard wells: Add **10 µl Standard** into Standard wells.
3. Add **200 µl Reagent Dye** to each well.
4. Mix well. Incubate at **RT** for **2 min**. Read the OD at **595nm**

Reagent	Sample	Standard	Blank
Sample	10 µl	-	-
Standard	-	10 µl	-
Distilled water	-		10 µl
Reagent Dye	200 µl	200 µl	200 µl
Mix well. Incubate at RT for 2 min. Read the OD at 595nm			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

2. Calculation:

A. Definition:

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

W: the weight of sample, g;

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

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

V_{assay}: the volume of Assay Buffer III, 500 µl = 0.5 ml.

B. Formula:

a). According to the weight concentration of sample

Hordein activity (mg /g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample)} - OD_{Sample})] / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{assay)}]$

$=4X (OD_{Sample} - OD_{Sample}) / [(OD_{Standard} - OD_{Blank}) X W]$

3. Detection range:

The detection range is from 0.02 mg/ml – 2 mg/ml.

4. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serially diluted standards are necessary for this kit.