Indoleacetic Acid OxidaseAssay Kit

ARG83428 Indoleacetic Acid Oxidase Assay Kit can be used to measure Indoleacetic Acid Oxidase in tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83428

Package: 96 wells

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INTRODUCTION

Indoleacetic Acid Oxidase (IAAO), an enzyme that oxidizes and breaks down indoleacetic acid in plants. An iron-containing hemoglobin, manganese and monohydric phenols are required as cofactors. The final products of oxidation are physiologically inactive 3-methyleneoxindole and 3-methyloxindole. The shoot and root tips contain less IAA oxidase than older tissues. The further away from the shoot or root tip, the higher the enzyme activity. In dwarf plants, the activity of IAA oxidase is high, which restricts the growth of plants and shows the characteristics of dwarf.

PRINCIPLE OF THE ASSAY

Indoleacetic Acid OxidaseAssay Kit determined Indoleacetic Acid Oxidaseactivity in tissue extracts, cell lysate, cell culture media and other biological fluids.. The Indoleacetic Acid Oxidaseactivity can be measured at a colorimetric readout at 530 nm

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Assay Buffer	4 X 30 ml	4°C
Substrate	1 vial (lyophilized)	4°C
Reagent Dye	1 vial (lyophilized)	4°C
Reagent Dye Diluent	10 ml	4°C
Reaction Buffer	1 vial (lyophilized)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 530 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.
- Store all component at 4°C.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- 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.
- Avoid using reagents from different batches.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.

SAMPLE COLLECTION & STORAGE INFORMATION

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

For tissue - Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 4000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep on ice for detection.

Note: For other liquid sample, it can be assayed directly.

REAGENT PREPARATION

- **Reaction Buffer:** Reconstitute the Reaction Buffer with **7 ml** of **Assay Buffer**. Allow the Reaction Buffer keep on bench for few minutes. Make sure the Reaction Buffer is dissolved completely and mixed thoroughly before use.

- **Substrate:** Reconstitute the Substrate with **2 ml** of **Assay Buffer**. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.

- **Reagent Dye:** Reconstitute the Reagent Dye with **10 ml** of **Reagent Dye Diluent.** Allow the Substrate keep on bench for few minutes. Make sure the Reagent Dye is dissolved completely and mixed thoroughly before use.

- **Standard:** Reconstitute the Standard with **1 ml** of **Assay Buffer, heat at 50 °C to dissolve** before use. Then add **100 µl ml Standard Buffer** into **100 µl Assay Buffer**, the concentration will be 2 µmol/ml.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **10 µl Sample** into Sample wells.
2. Add **20 µl Standard** into Standard wells.
3. Add **70 µl Reaction Buffer** into all wells.
4. Add **20 µl Substrate** into Sample and Control wells.
5. Adjust to the volume to **100µl** with **Distilled water**
6. Mix well. Incubate at **30°C** for **30 min**.
7. Add **100 µl Reagent Dye** into all wells.
8. Mix well. Incubate at**30°C** for **30 min**. Read the OD at **530 nm**.

Summary of Indoleacetic Acid Oxidase Assay Procedure

Reagent	Sample	Standard	Blank	Control
Sample	10 µl	-	-	
Standard	-	20 µl	-	-
Reaction Buffer	70 µl	70 µl	70 µl	70 µl
Substrate	20 µl	-	-	20 µl
Distilled water	-	10 µl	30 µl	10 µl
Mix well. Incubate at 30°C for 30 min.				
Reagent Dye	100 µl	100 µl	100 µl	100 µl
Mix well. Incubate at 30°C for 30 min. Read the OD at 530 nm.				

CALCULATION OF RESULTS

1. Unit Definition: One unit Indoleacetic Acid Oxidaseactivity is defined as decomposes 1 µmol of indoleacetic acid per hour in the reaction system.

2. Calculate the average absorbance values for each set of samples and control.

3. Calculation:

A. Definition:

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the concentration of Standard, 2 µmol/ml;

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

$V_{Standard}$: the volume of reaction Standard, 20 µl = 0.02 ml;

V_{assay}: the volume of Assay Buffer, 1000 µl = 1 ml;

W: the weight of sample, g;

T: the reaction time, 30 minutes.

B. Formula:

a). According to the protein concentration of sample

Indoleacetic Acid Oxidaseactivity (U/mg) =

$(OD_{Control} - OD_{Sample}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample}) \times T]$

$= 8 \times (OD_{Control} - OD_{Sample}) / [(OD_{Standard} - OD_{Blank}) \times C_{Protein}]$

b). According to the weight of sample

Indoleacetic Acid Oxidaseactivity (U/mg) =

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

$= 8 \times (OD_{Control} - OD_{Sample}) / [(OD_{Standard} - OD_{Blank}) \times W]$

c). According to the volume of sample

Indoleacetic Acid Oxidaseactivity (U/ml) =

$(OD_{Control} - OD_{Sample}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T]$

$= 8 (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank})$