Indoleacetic Acid Oxidase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1253

(Version 1.3A)

Detection and Quantification of Indoleacetic Acid Oxidase(IAAO) Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Indoleacetic Acid Oxidase (IAAO), an enzyme that oxidizes and breaks down indoleacetic acid in plants.An iron-containing hemoglobin, manganese and monohydric phenolsare 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. Indoleacetic Acid Oxidase ActivityColorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of indoleacetic acid oxidase activity in a variety of samples. The intensity of the product color, measured at 530 nm, is inversely proportional to the indoleacetic acid oxidase activity in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	Powder x 1	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	10 mlx 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 530 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven
9. Ice

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml Assay Bufferand heat at 50 °C to dissolve to generate 20 mmol/L of standardstock solution.Keep in dark and store at 4 °C for 3 days or -20°C for 1-2months after reconstitution.Then dilute to 2mmol/Lstandard top solutionby adding 100 µlstock solution into 900 µl Assay Buffer. Perform 2-fold serial dilutions of the top standardsolution using Assay Bufferto make the standard curve. The concentration of standard curve could be 2/1/0.5/0.25/0.125/0.0625/0.0312mmol/L.

Reaction Buffer: Add 7 ml Assay Buffer to dissolve before use.Keep in dark and store at 4 °C for 3-4 days or -20°C for 1-2months after reconstitution.

Substrate: Briefly centrifuge prior to opening. Add 2 ml Assay Buffer to dissolve before use. Keep in dark and store at 4 °C for 3 days or -20°C for 1-2months after reconstitution.

Dye Reagent: Add 10 ml Dye Reagent Diluent to dissolve before use.Keep in dark and store at 4 °C for 3-4months after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For tissue samples

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

2. For liquid samples

Detect directly, or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Standard	Blank
Sample	10 µl	--	--	--
Standard	--	--	20 µl	--
Distilled water	--	10 µl	10 µl	30 µl
Reaction Buffer	70 µl	70 µl	70 µl	70 µl
Substrate	20 µl	20 µl	--	--
Mix, put it into the convection oven,incubate at 30°C for 30 minutes.				
Dye Reagent	100µl	100µl	100µl	100µl
Mix,put it into the convection oven,30 °C for 30 minutes, record absorbance measured at 530 nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**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

Unit Definition: One unit ofIndoleacetic Acid Oxidase activity is defined as the enzyme decomposes1 μmol of indoleacetic acid per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Control}-OD_{Sample}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Control}-OD_{Sample})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times C_{Protein}\times T}(U/mg)$$

2.2 According to the weight of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Control}-OD_{Sample})}{(OD_{Standard}-OD_{Blank}) \times W \times (V_{Sample}/V_{Assay})\times T}(U/g)$$

2.3 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Control}-OD_{Sample})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

$C_{Standard}$: the concentration of standard, mmol/L= μmol/mL

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

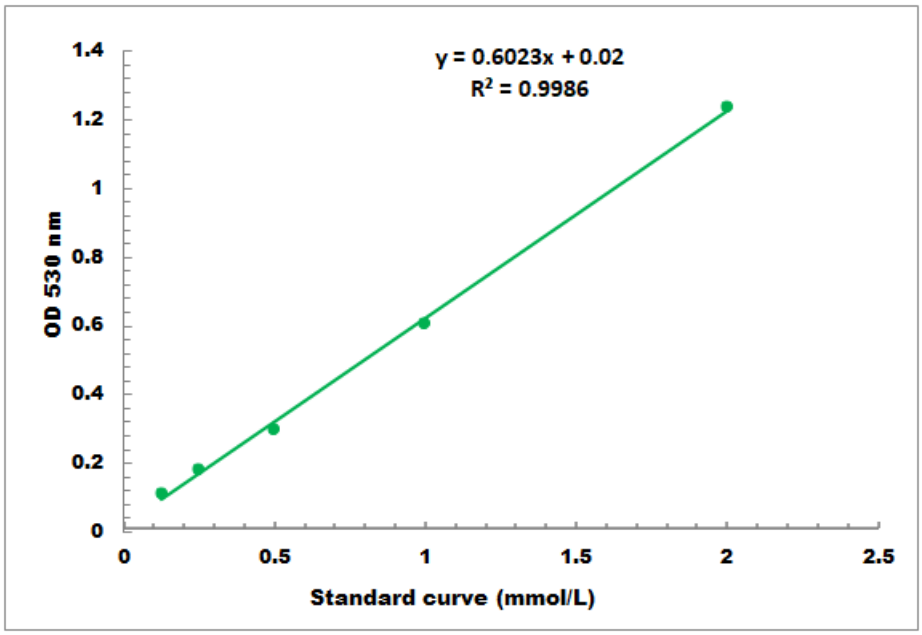
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L -2 mmol/L