Indoleacetic Acid Oxidase

Microplate Assay Kit

User Manual

Catalog # CAK1253

(Version 1.2A)

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 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. Indoleacetic Acid Oxidase 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 ml x 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 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Reaction Buffer: add 7 ml Assay Buffer to dissolve before use.

Substrate: add 2 ml Assay Buffer to dissolve before use.

Dye Reagent: add 10 ml Dye Reagent Diluent to dissolve before use.

Standard: add 1 ml Assay Buffer, heat at 50 °C to dissolve before use; then add 100 µl into 900 µl Assay Buffer, mix, the concentration will be 2 mmol/L.

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. SAMPLE PREPARATION

1. For tissue samples

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 it on ice for detection.

2. For liquid samples

Detect directly, or dilute with Assay Buffer.

V. ASSAY PROCEDURE

Add following reagents into the 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	100 µl	100 µl	100 µl	100 µl
Mix, put it into the convection oven, 30 °C for 30 minutes, record absorbance measured at 530 nm.				

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of Indoleacetic Acid Oxidase activity is defined as the enzyme decomposes 1 μmol of indoleacetic acid per minute.

1. According to the protein concentration of sample

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

$$= 0.133 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the volume of sample

$$IAAO\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.133 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank})$$

3. According to the weight of sample

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

$$= 0.133 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the standard concentration, 2 mmol/L = 2 μmol/ml;

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

W: the weight of sample, g;

$V_{Standard}$: the volume of standard, 0.02 ml;

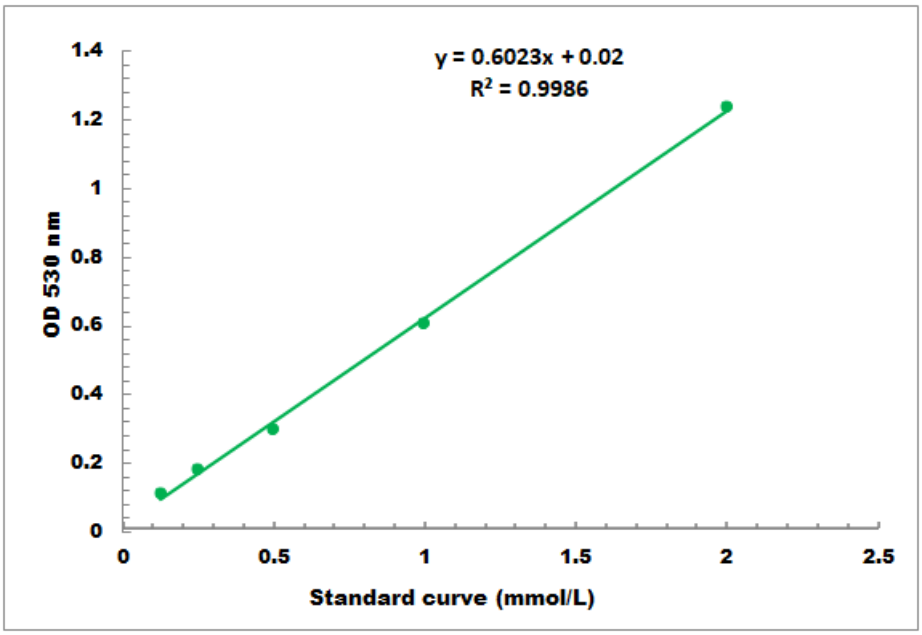
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of assay buffer, 1 ml;

T: the reaction time, 30 minutes.

VII. 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES