Aniline 4-Hydroxylase

Microplate Assay Kit

User Manual

Catalog # CAK1032

(Version 1.2C)

Detection and Quantification of Aniline 4-Hydroxylase (AH) Activity in Tissue extracts, Cell lysate Samples.

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

I. INTRODUCTION

Cytochrome P450 enzymes play an important role in the metabolism of exogenous substrate, especially drugs and poisons. As an important enzyme of P450 family, Aniline 4-Hydroxylase (AH) is equivalent to isoform CYP2E1. CYP2E1 not only participate the drug metabolism, but also catalytic the activation of variety of precarcinogens and prepoison.

AH catalytic the hydroxylation of aniline, and further convert to pheno-indole compound, which has a characteristic absorption peak at 630nm; AH activity was calculate by measuring the absorbance change rate at 630nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 4	4 °C
Assay Buffer II	30 ml x 2	4 °C
Substrate I	Powder x 1	4 °C, keep in dark
Substrate II	Powder x 1	4 °C
Substrate Diluent	1 ml x 2	4 °C
Stop Solution	10 ml x 1	4 °C
Dye Reagent I	Powder x 1	4 °C, keep in dark
Dye Reagent II	Powder x 1	4 °C
Standard	Powder x 1	4 °C, keep in dark
Standard Diluent	2 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Substrate I: add 1 ml Substrate Diluent to dissolve before use, store at 4 °C.

Substrate II: add 1 ml Substrate Diluent to dissolve before use, store at 4 °C.

Dye Reagent I: add 5 ml distilled water to dissolve before use, store at 4 °C.

Dye Reagent II: add 5 ml distilled water to dissolve before use, store at 4 °C.

Standard: add 1 ml Standard Diluent to dissolve, mix; then add 2 µl into 498 µl Standard Diluent, mix, store at 4 °C. The concentration will be 100 µmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 630 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.5 g tissue, homogenize with 1 ml Assay Buffer I on ice, centrifuged at 10,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube. Centrifuged at 100,000g 4 °C for 60 minutes, discard the supernatant. Add 1 ml Assay Buffer I to the precipitation, mix and vortex, centrifuged at 100,000g 4 °C for 30 minutes, discard the supernatant. Add 0.5 ml Assay Buffer II to the precipitation, mix and vortex. Keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank
Sample	10 µl		--	--
Distilled water	--	10 µl	--	--
Assay Buffer II	70 µl	70 µl	--	--
Substrate I	10 µl	10 µl	--	--
Substrate II	10 µl	10 µl	--	--
Mix, put it in the oven, 37 °C for 30 minutes.				
Stop Solution	100 µl	100 µl	--	--
Mix, put them on ice for 5 minutes. Centrifuged at 8,000g at room temperature for 5 minutes, take the supernatant into the microplate.				
Supernatant	100 µl	100 µl	--	--
Standard	--	--	100 µl	--
Distilled water	--	--	--	100 µl
Dye Reagent I	50 µl	50 µl	50 µl	50 µl
Dye Reagent II	50 µl	50 µl	50 µl	50 µl
Mix, keep at room temperature for 30 minutes, then record absorbance measured at 630 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 AH activity is the enzyme that generates 1 μmol of 4-aminophenol per minute.

1. According to the protein concentration of sample

$$AH\ (U/mg) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) \times 2 / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.00667 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$AH\ (U/g) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) \times 2 / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.01333 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the standard concentration, 100 μmol/L = 0.1 μmol/ml;

$V_{Standard}$: the volume of standard, 0.1 ml;

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

W: the weight of sample, g;

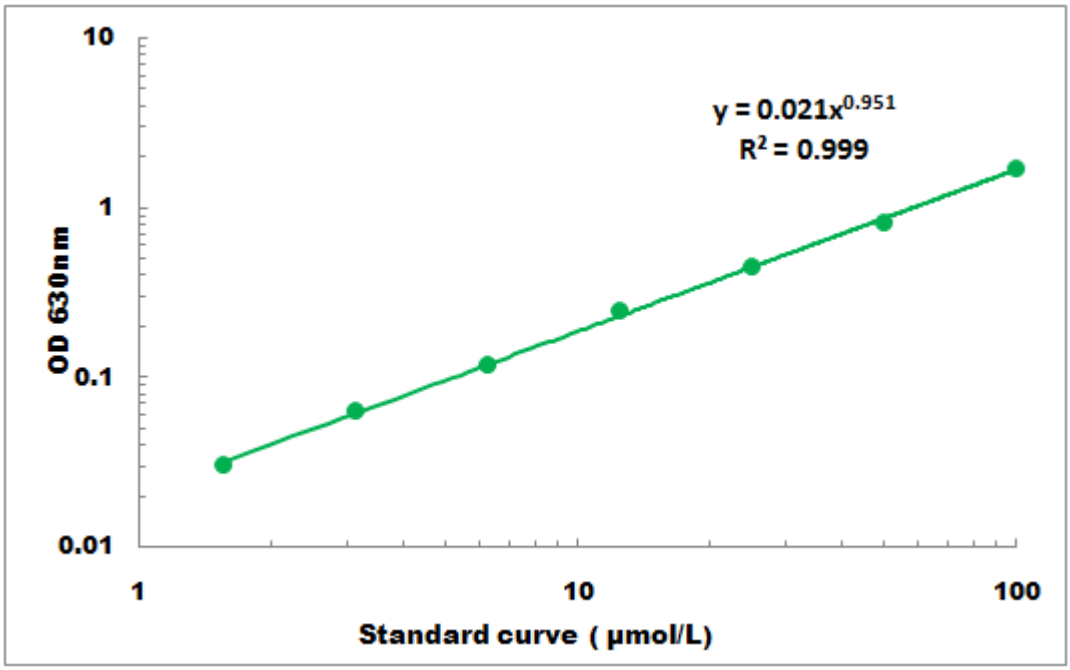
V_{Sample}: the volume of sample, 0.1 ml;

V_{Assay}: the volume of Assay Buffer II, 0.5 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: 1 μmol/L - 100 μmol/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