



Aniline 4-Hydroxylase Microplate Assay Kit User Manual

Catalog # ASK1032

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.

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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
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 μ l		--	--
Distilled water	--	10 μ l	--	--
Assay Buffer II	70 μ l	70 μ l	--	--
Substrate I	10 μ l	10 μ l	--	--
Substrate II	10 μ l	10 μ l	--	--
Mix, put it in the oven, 37 °C for 30 minutes.				
Stop Solution	100 μ l	100 μ l	--	--
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 μ l	100 μ l	--	--
Standard	--	--	100 μ l	--
Distilled water	--	--	--	100 μ l
Dye Reagent I	50 μ l	50 μ l	50 μ l	50 μ l
Dye Reagent II	50 μ l	50 μ l	50 μ l	50 μ l
Mix, keep at room temperature for 30 minutes, then record absorbance measured at 630 nm.				

VI. CALCULATION

Unit Definition: One unit of AH activity is the enzyme that generates 1 nmol of 4-aminophenol per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{AH (U/mg)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times 2 / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 6.67 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{AH (U/g)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times 2 / (V_{\text{Sample}} \\ &\quad \times W / V_{\text{Assay}}) / T \\ &= 13.33 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

C_{Standard} : the standard concentration, 100 $\mu\text{mol/L}$ = 100 nmol/ml;

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

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

W : the weight of sample, g;

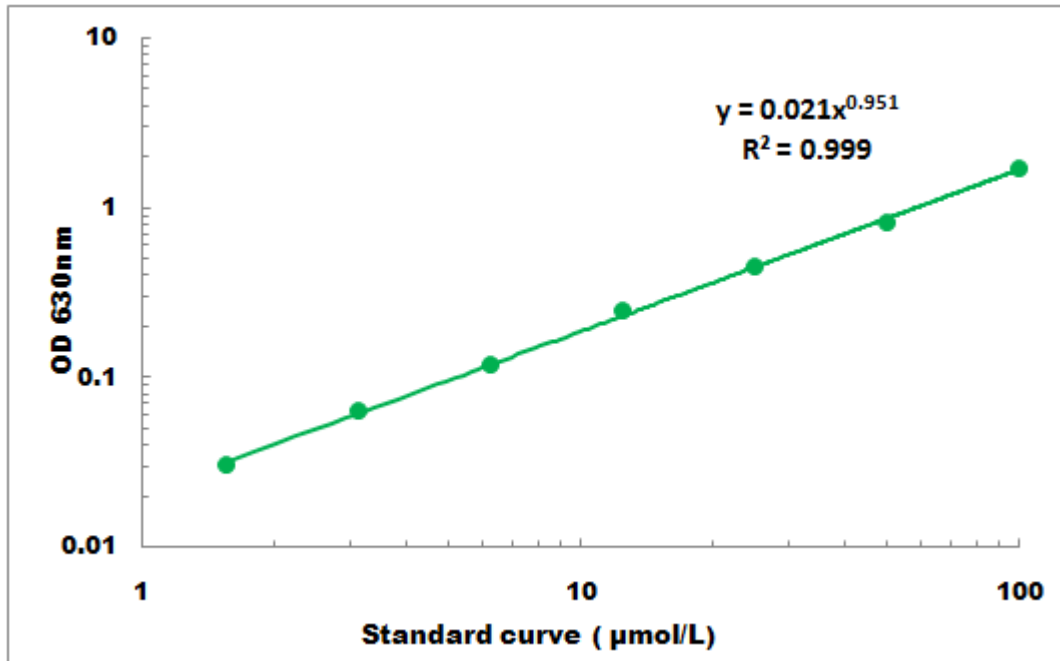
V_{Sample} : the volume of sample, 0.1 ml;

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