Aniline 4-Hydroxylase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1032

(Version 1.3C)

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.

Aniline 4-Hydroxylase Activity ColorimetricMicroplate Assay Kit provides a simple and direct procedure for measuringAniline 4-Hydroxylase activity in a variety of samples.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 mlx 4	4 °C
Assay Buffer II	30 mlx 2	4 °C
Substrate I	Powderx 1	4 °C, keep in dark
Substrate II	Powderx 1	4 °C
Substrate Diluent	1 mlx 2	4 °C
Stop Solution	10 mlx 1	4 °C
Dye Reagent I	Powderx 1	4 °C, keep in dark
Dye Reagent II	Powderx 1	4 °C
Standard	Powderx 1	4 °C, keep in dark
Standard Diluent	2 mlx 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 630 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml Standard Diluentto generate 25 mmol/L of standardstock solution, store at 4 °C for 1 day or -20°C for 3daysafter reconstitution.Then dilute to 0.1 mmol/Lstandard top solutionby adding 2 µlstock solution into 498µlStandard Diluent**.** Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 100/50/25/12.5/6.25/3.12/1.56µmol/L.

Substrate I: Briefly centrifuge prior to opening. Add 1 mlSubstrate Diluent to dissolve before use.Keep in dark for immediate use or store at -80°C for 1-2weeks after reconstitution.

Substrate II: Briefly centrifuge prior to opening. Add 1 mlSubstrate Diluent to dissolve before use. Keep in dark for immediate use or store at -80 °C for 2-4 weeks.

Dye Reagent I: Add 5 mldistilled water to dissolve before use. Keep in dark and store at 4 °C for 1 week or -20°C for 1 month after reconstitution.

Dye Reagent II: Add 5 mldistilled water to dissolve before use, store at 4 °C.Store at 4 °C for 2-3months after reconstitution.

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

V. SAMPLE PREPARATION

1.For tissue samples

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

VI. 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 30minutes.				
Stop Solution	100 µl	100 µl	--	--
Mix, put them on icefor 5minutes.Centrifuged at 8,000g at room temperature for 5minutes, 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 30minutes, then record absorbance measured at 630 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 of AH activity is the enzyme that generates 1 μmol of 4-aminophenol per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.3 According to the weight of sample

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

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, μmol/L

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

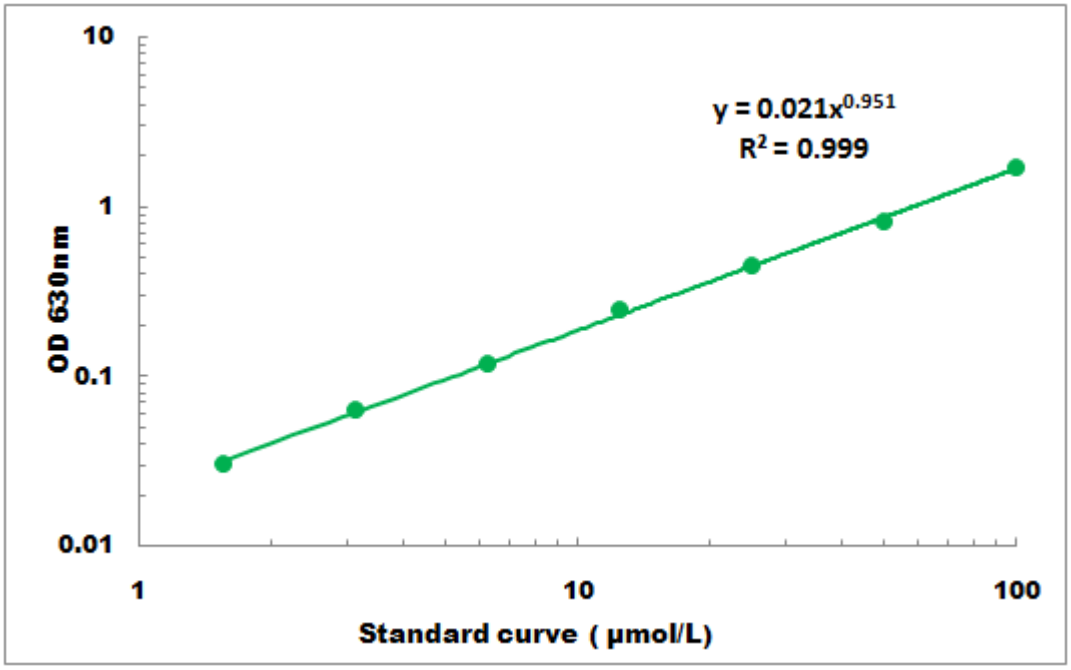
V_{Sample}: the volume of sample in assay procedure, mL

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