



Aminopyrine N-demethylase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1031

(Version 1.4C)

Detection and Quantification of Aminopyrine N-demethylase(AND)
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, Aminopyrine N-demethylase (AND) is equivalent to isoform CYP3A4 and closely related to the methylation reaction of drugs.

Aminopyrine N-demethylase Activity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring Aminopyrine N-demethylase activity in a variety of samples. AND catalytic aminopyrine release formaldehyde, formaldehyde content was measured by colorimetry Nash, then AND activity can be calculated.

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 1	4 °C
Stop Solution	10 mlx 1	4 °C
Dye Reagent	10 mlx 1	4 °C, keep in dark
Standard (50µmol/L)	1 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 420 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. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 50/25/12.5/6.25/3.12/1.56/0.78 $\mu\text{mol/L}$.

Substrate I: add 1 ml Substrate Diluent to dissolve before use. Keep in dark and store at 4 °C for 2-3 weeks or -20°C for 3 months after reconstitution.

Substrate II: add 1 ml distilled water to dissolve before use. Keep in dark for immediate use or store at -80 °C for 2-4 weeks.

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

V. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.5g 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.

2. For liquid samples

Detect it directly.

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 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	100 µl	100 µl	100 µl	100 µl
Mix, put it in the oven, 60 °C for 10 minutes, then put it on ice immediately. Record absorbance measured at 420 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 AND activity is defined as the enzyme generates 1 μmol of formaldehyde per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.1 According to the weight of sample

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

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

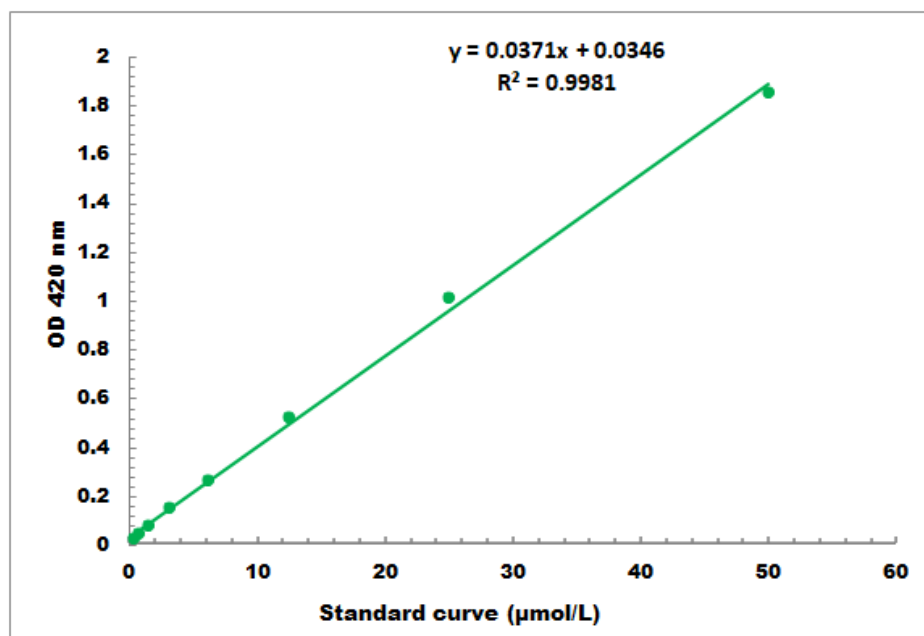
V_{Sample}: the volume of sample in assay procedure, mL

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: 0.5 μmol/L - 50 μmol/L