



**Aminopyrine N-demethylase
Microplate Assay Kit
User Manual**

Catalog # CAK1031

(Version 1.3C)

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.

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 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 1	4 °C
Stop Solution	10 ml x 1	4 °C
Dye Reagent	10 ml x 1	4 °C, keep in dark
Standard (50 µmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

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

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

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. 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.

2. For liquid samples

Detect it directly.

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

1. According to the protein concentration of sample

$$\begin{aligned} \text{AND (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) / T \times 2 \\ &= 0.00333 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{AND (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times \\ &\quad W / V_{\text{Assay}}) / T \times 2 \\ &= 0.00667 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

C_{Standard} : the standard concentration, 50 $\mu\text{mol/L}$ = 0.05 $\mu\text{mol/ml}$;

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

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

W: the weight of sample, g;

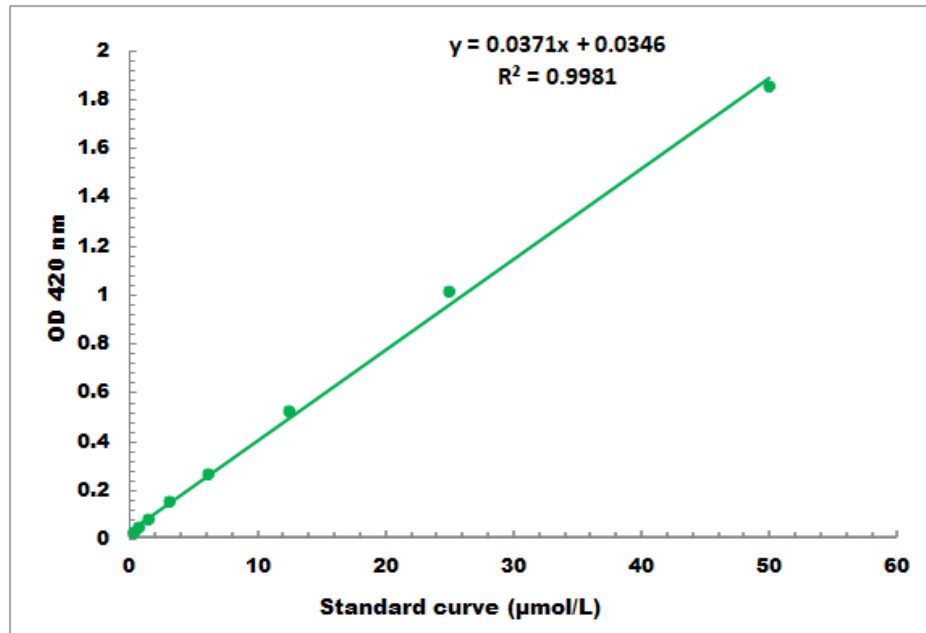
V_{Sample} : the volume of sample, 0.1 ml;

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: 0.5 µmol/L - 50 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