Aniline 4-Hydroxylase (AH) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC2745

Size:100T/48S

Components:

Reagent I: Powder×2. Store at 2-8°C. Add 60mL distilled water to each Reagent I and mix well before use. It can be stored at 2-8°C for four weeks.

Reagent II: Liquid 30mL×1. Store at 2-8°C.

Reagent III: Powder×2. Store at 2-8°C. Add 6mL distilled water to each Reagent III and mix well before use. It can be stored at -20°C for two weeks after dispensing to avoid repeated freezing and thawing.

Reagent IV: Powder×1. Store at 2-8°C. Add 3mL distilled water and mix well before use. It can be stored at 2-8°C for four weeks.

Reagent V: Liquid 11mL×1. Store at 2-8°C.

Reagent VI: Powder×2. Store at 2-8°C. Add 6mL distilled water to each Reagent VI and mix well before use. It can be stored at 2-8°C for four weeks.

Reagent VII: Liquid 12mL×1. Store at 2-8°C.

Standard: Liquid 1mL×1, 10nmol/mL of standard solution, store at 2-8°C.

Product Description:

Cytochrome P450 enzymes are a group of isoenzymes mainly found in the liver. They play an important role in the metabolism of exogenous substances, especially the metabolism of drugs and poisons. Aniline 4-Hydroxylase (AH) is one kind of CYP2E1 isomers in the P450 enzymes family. CYP2E1 not only participate the drug metabolism, but also catalytic the activation of variety of procarcinogens and prepoison.

AH catalyzes the hydroxylation of aniline to produce 4-aminophenol, and further convert to pheno-indole compound, which has a characteristic absorption peak at 630nm. AH activity is calculated by measuring the rate of increase in absorbance at 630nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, constant temperature foster box/water-bath, desk centrifuge, transferpettor, micro glass cuvette/96-well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure:

I. Sample preparation

1. Remove cell nucleus and mitochondria: weigh about 0.5g of tissue, add 1 mL of pre-cooled reagent I, grind thoroughly on ice, centrifuge at 10000g at 4°C for 30 minutes, take the supernatant, and transfer to an ultracentrifuge tube.
2. Crude microsomes: centrifuge for 60 min at 4°C, 100 000 g, and discard the supernatant.

3. Removal of impurities such as hemoglobin: add 1 mL of reagent I to the precipitate of step 2, and then fully shake to dissolve it after it is tightly capped. Centrifuge at 100 000 g for 30 min, and discard the supernatant.
4. Final microsomes: add 0.5 mL of reagent II to the precipitation of step 3, cover tightly and shake to dissolve it, and store at 4°C for testing.

II. Determination

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 630 nm and set spectrophotometer counter to zero with distilled water.
2. Keep Reagent V on ice for 30 min.
3. Add reagents according to the following table:

Reagent (μL)	Contrast tube (A_C)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	50	50	-	-
Reagent III	100	100	-	-
Reagent IV	-	50	-	-
Distilled Water	50	-	-	-
Mix well, incubate accurately at 37°C for 30min			-	-
Reagent V	100	100	-	-
Mix well, cool on ice for 5min; centrifuge for 10 min at 4°C, 11000rpm and take the supernatant			-	-
Supernatant	100	100	-	-
Standard	-	-	100	-
Distilled Water	-	-	-	100
Reagent VI	100	100	100	100
Reagent VII	100	100	100	100

Mix well and stand at room temperature for 30min. Take 200μL reaction solution and measure the absorbance at 630nm, record A_C, A_T, A_S, A_B. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. A contrast tube is required for each test tube and the standard tube and blank tube need only be tested once or twice.

III. Calculation:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes production of 1 nmol 4-aminophenol in the reaction system per minute at 37°C every mg protein.

AH activity (U/mg prot) $= C_S \times V_T \times \Delta A_T \div \Delta A_S \div (Cpr \times Vs) \div T = 2 \times \Delta A_T \div \Delta A_S \div Cpr$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes production of 1 nmol 4-aminophenol in the reaction system per minute at 37°C every g sample.

AH activity (U/g weight) $= C_S \times V_T \times \Delta A_T \div \Delta A_S \div (W \times Vs \div Ve) \div T = \Delta A_T \div \Delta A_S \div W$

C_S: Content of standard, 10nmol/mL;

V_T: Total supernatant volume, 0.3mL;
Cpr: Crude enzyme protein concentration, mg/mL;
Vs: Crude enzyme volume, 0.05 mL;
T: Reaction time, 30 min;
W: Sample weight, g;
Ve: Extract volume of reagent II, 0.5mL.

Note:

1. If ΔA_T is close to ΔA_B or ΔA_T is low, it is recommended to increase the sample size before determination. If $A_T > 1.5$ or $\Delta A_T > 1$, it is recommended to dilute the sample with Reagent II before determination. And modify the calculation formula.
2. After the extraction of crude enzyme solution, the determination should be completed on the same day.