（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F023

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=390 nm/468 nm)

Detection range：0.29-4.42 U/L

Elabscience® Cytochrome P450 2D6 (CYP2D6) Activity Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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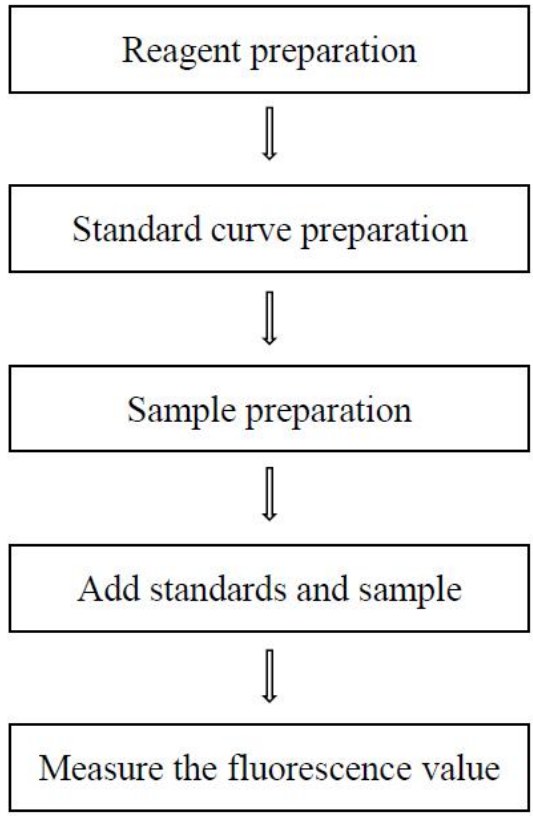
Assay summary



Intended use

This kit can be used to measure cytochrome P450 2D6 (CYP2D6) activity in liver microsomes samples.

Detection principle

Cytochrome P450 2D6 (CYP2D6) is a member of the cytochrome P450 enzyme family that is responsible for metabolizing approximately 25% of drugs in the human body and is primarily expressed in the liver. CYP2D6 has significant metabolic effects on many clinically important drugs, including antidepressants, antipsychotics, beta-blockers, and painkillers. It converts these drugs into more excretable metabolites through oxidation reactions. The key role of CYP2D6 in drug metabolism and the significant influence of individual differences make detecting the activity of this enzyme important for personalized medicine.

The detection principle of this kit: the substrate is converted into a fluorescent substance after catalysis by CYP2D6, and the fluorescence value is detected at the excitation wavelength of 390 nm and the emission wavelength of 468 nm, and the enzyme activity is calculated by the fluorescence value of the standard product.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months shading light
Reagent 2	Extraction Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months shading light
Reagent 3	Diluent Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months shading light
Reagent 4	Substrate	0.02 mL × 2 vials	0.02 mL × 4 vials	-20°C, 12 months shading light
Reagent 5	Reducing Reagent	0.5 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months shading light
Reagent 6	Enzyme Reagent	0.25 mL × 1 vial	0.5 mL × 1 vial	-20°C, 12 months shading light
Reagent 7	5 mmol/L Standard Solution	0.5 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=390 nm/468 nm), Incubator

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of substrate working solution:

Take a vial of substrate, centrifuge at 500×g for 3 min at 25℃, add 1 mL of buffer solution and mix well. The substrate working solution should be prepared on spot protected from light and used up with the same day.

③ The preparation of measuring working solution:

Before testing, please prepare sufficient measuring working solution according to test wells. For example, prepare 250 μL of measuring working solution (mix well 220 μL of buffer solution, 25 μL of reducing reagent and 5 μL of enzyme reagent). The measuring working solution should be prepared on spot protected from light and used up with the same day.

④ The preparation of 200 μmol/L standard solution:

Before testing, please prepare sufficient 200 μmol/L standard solution according to test wells. For example, prepare 1000 μL of 200 μmol/L standard solution (mix well 40 μL of 5 mmol/L standard solution and 960 μL of buffer solution). The 200 μmol/L standard solution should be prepared on spot protected from light and used up with the same day.

⑤ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 200 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 40, 60, 80, 120, 140, 160, 200 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**40**	**60**	**80**	**120**	**140**	**160**	**200**
200 μmol/L Standard (μL)	0	40	60	80	120	140	160	200
Buffer solution (μL)	200	160	140	120	80	60	40	0

Sample preparation

① Sample preparation

The preparation of liver microsomes samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 50 mg).

② Homogenize 50 mg tissue in 450 μL of extraction solution with a dounce homogenizer at 4℃.

③ Centrifuge at 7800×g for 20 min at 4℃ to collect supernatant.

④ Centrifuge at 21000×g for 45 min at 4℃ to remove supernatant.

⑤ Add 900 μL of diluent solution into the insoluble material, mix well to get 10% liver microsomes sample.

⑥ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver microsomes	1

Note: The diluent is diluent solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 20 μL of standard with different concentrations into the well.

Sample well: add 20 μL of sample into the well.

② Add 30 μL of substrate working solution into each well.

③ Add 50 μL of measuring working solution into each well.

④ Mix fully with fluorescence microplate for 5 s. Measure the fluorescence intensity of sample wells at the excitation wavelength of 390 nm and the emission wavelength of 468 nm, as F_1.

⑤ Incubate at 37°C for 45 min protected from light. Measure the fluorescence intensity at the excitation wavelength of 390 nm and the emission wavelength of 468nm, as F_2. (The standard curve is fitted to the standard well in F_2)

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean F_2 value of the blank (Standard #①) from all standard readings. This is the absoluted F_2 value.

3. Plot the standard curve by using absoluted F_2 value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Liver microsomes samples:

Definition: The amount of enzyme in 1 g liver microsomes protein per 1 min that produce 1 μmol product at 37 °C is defined as 1 unit.

$$\text{CYP2D6 activity (U/gprot)} = (\Delta F - b) \div a \div T \times f \div C_{pr}$$

[Note]

ΔF: $\Delta F = F_2 - F_1$.

T: Reaction time, 45 min.

C_{pr}: Concentration of protein in sample, gprot/L

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver microsomes were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.50	1.30	2.80
%CV	2.2	4.9	3.2

Inter-assay Precision

Three mouse liver microsomes were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.50	1.30	2.80
%CV	6.4	9.4	8.6

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 101%.

	Sample 1	Sample 2	Sample 3
Expected Conc(U/L)	0.50	1.30	2.80
Observed Conc(U/L)	0.495	1.326	2.828
Recovery rate (%)	99	102	101

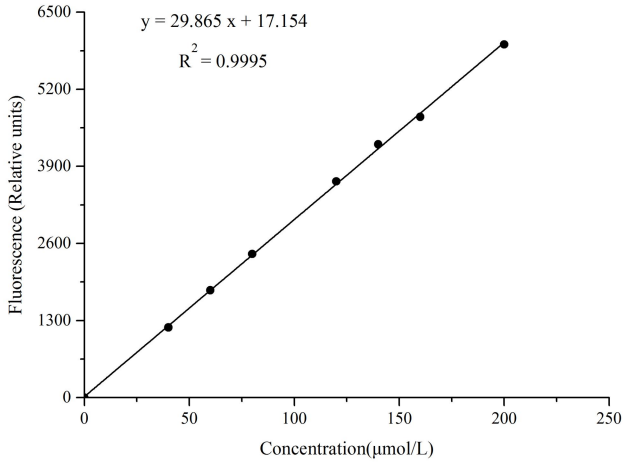
Sensitivity

The analytical sensitivity of the assay is 0.29 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**40**	**60**	**80**	**120**	**140**	**160**	**200**
F_2 value	208	1416	2052	2646	3857	4533	5008	6225
	216	1377	1994	2623	3862	4435	4888	6115
Average F_2 value	212	1397	2023	2634	3859	4484	4948	6170
Absoluted F_2 value	0	1184	1811	2422	3647	4272	4735	5958



Appendix II Example Analysis

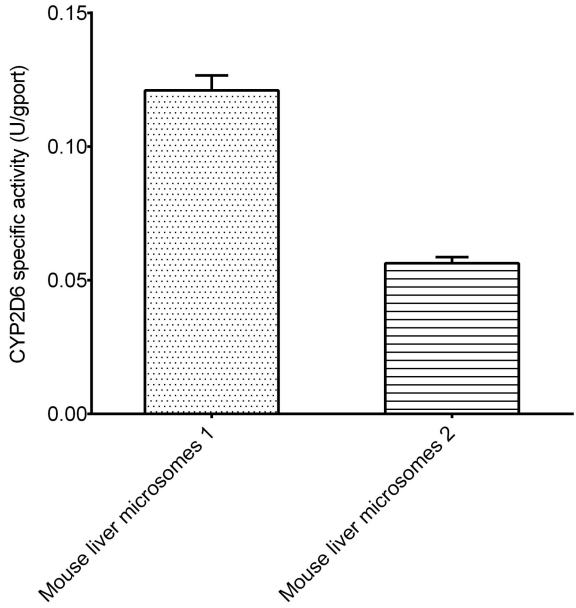
Example analysis:

Take 20 μL of 10% mouse liver microsomes and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 29.865 x + 17.154, the average F_1 value of the sample well is 317, the average F_2 value of the sample well is 1463, $\Delta F = F_2 - F_1 = 1463 - 317 = 1146$, the concentration of protein is 6.94 gprot/L, and the calculation result is:

CYP2D6 activity (U/gprot) = (1146 – 17.154) ÷ 29.865 ÷ 45 ÷ 6.94 = 0.121 U /gprot

Detect 10% mouse liver microsomes 1 (the concentration of protein is 6.94 gprot/L) and 10% mouse liver microsomes 2 (the concentration of protein is 14.88 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.