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

Catalog No：E-BC-K808-M

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

Measuring instrument：Microplate reader（430 - 470 nm）

Detection range：9.11-550 U/L

Elabscience® Cytochrome P450 Reductase(CPR) Activity Assay Kit

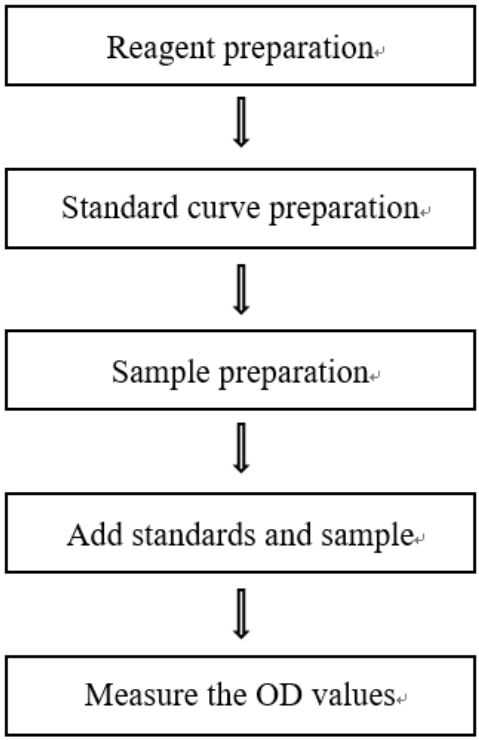
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

This kit can be used to measure the activity of cytochrome P450 reductase(CPR) in tissue and cell samples.

Detection principle

Cytochrome P450 reductase (CPR) is an important part of the cytochrome P450 (CYPs) system. After obtaining electrons from the electron donor, CPR transfers electrons to CYPs, which react with oxidized substrates and molecules to form reduced substrates and water.

This kit uses the electron transfer characteristics of CPR to catalyze the substrate to generate chromophore, which has a maximum absorption at 450 nm. The activity of CPR can be calculated by measureing the OD value at 450 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Control Solution	3.5 mL ×1 vial	7 mL ×1 vial	-20℃, 12 months, shading light
Reagent 2	Enzyme Diluent	12.5 mL ×1 vial	25 mL ×1 vial	-20℃, 12 months, shading light
Reagent 3	Substrate	Power ×2 vials	Power ×4 vials	-20℃, 12 months, shading light
Reagent 4	Standard	Power ×2 vials	Power ×2 vials	-20℃, 12 months, shading light
	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:

Micropipettor, Vortex mixer, Centrifuge, Microplate reader (430-470 nm, optimum wavelength: 450 nm), 37℃ incubator

Reagents:

Normal Saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of substrate working solution：

Dissolve one vial of substrate with 6 mL of enzyme diluent, mix well to dissolve. Store at 2-8 ℃ for 3 days protected from light.

③ Preparation of 1 mmol/L standard solution：

Dissolve one vial of standard with 5 mL of double distilled water, mix well to dissolve. Store at -20 ℃ for 7 days.

④ Preparation of 500 μmol/L standard solution：

Dissolve 500 μL of 500 μmol/L standard solution with 500 μL of double distilled water, mix well to dissolve. Store at -20 ℃ for 3 days.

⑤ The preparation of standard curve：

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

Dilute 500 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 100, 200, 250, 300, 350, 400, 500 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	0	100	200	250	300	350	400	500
500 μmol/L standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4 ℃.

④ Centrifuge at 10000 ×g for 10 min at 4 ℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ E-BC-K318-M is recommended for animal tissue samples. E-BC-K168-M is recommended for plant tissue samples.

Cell samples:

① Harvest the number of cells needed for each assay (initial recommendation 1 ×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1 ×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4 ℃.

④ Centrifuge at 10000 ×g for 10 min at 4 ℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ 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 tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1
1×10^6 CHO cells	1
1×10^6 Jurkat cells	1
1×10^6 293T cells	1
0.375×10^6 A549 cells	1
0.35×10^6 Hela cells	1
0.5×10^6 Molt-4 cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

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

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

Control well: add 20 μL of sample into the control wells.

② Add 200 μL of control solution into the standard wells.

③ Add 200 μL of substrate working solution into the sample wells.

④ Add 200 μL of double distilled water into control wells.

⑤ Mix fully for 5 s with microplate reader and incubate at 37 ℃ for 10 min protected from light. Measure the OD values of each well at 450 nm with microplate reader.

Note: If the control well OD of the pre-test sample is less than 0.05, the control well is not required for the formal experiment.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted OD 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:

Tissue and cell sample:

Definition: The amount of CPR in 1 g tissue or cell protein sample that consume 1 μmol NADPH in 1 minute at 37°C is defined as 1 unit.

$$\text{CPR content (μmol/gprot)} = (\Delta A_{450} - b) \div a \times f \div T \div C_{pr}$$

[Note]

ΔA_{450}: $OD_{Sample} - OD_{Blank}$

f: Dilution factor of sample before test.

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

T: Reaction time, 10 min.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver samples 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)	50.00	100.00	250.00
%CV	3.8	2.8	3.0

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	50.00	100.00	250.00
%CV	3.9	3.2	3.4

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 99%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (U/L)	150	270	360
Observed Conc. (U/L)	150.0	246.6	356.4
Recovery rate (%)	100	98	99

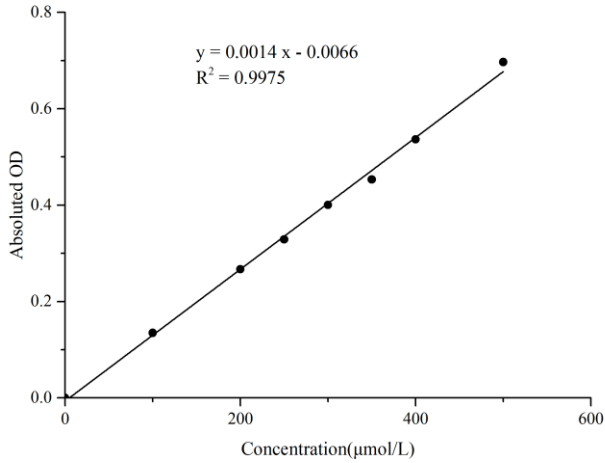
Sensitivity

The analytical sensitivity of the assay is 9.11 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 OD 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	100	200	250	300	350	400	500
OD value	0.165	0.305	0.438	0.497	0.571	0.615	0.676	0.868
	0.167	0.297	0.428	0.493	0.562	0.624	0.729	0.858
Average OD	0.166	0.301	0.433	0.495	0.567	0.620	0.703	0.863
Absoluted OD	0	0.135	0.267	0.329	0.401	0.454	0.537	0.697



Appendix II Example Analysis

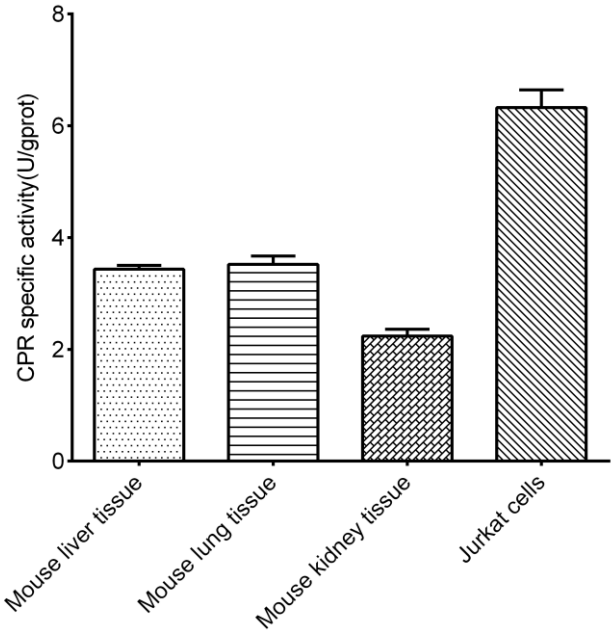
Example analysis:

Dilute 10% mouse liver tissue homogenate, take 20 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0014 x - 0.0066, the average OD value of the sample is 0.550, the average OD value of the control is 0.167, the concentration of protein in sample is 8.09 gprot/L, and the calculation result is:

$$\text{CPR activity (U/gprot)} = (0.550 - 0.167 + 0.0066) \div 0.0014 \div 8.09 \div 10 = 3.44 \text{ U/gprot}$$

Detect 10% mouse liver tissue homogenate (the concentration of protein is 8.09 gprot/L), 10% mouse lung tissue homogenate (the concentration of protein is 6.90 gprot/L), 10% mouse kidney tissue homogenate (the concentration of protein is 1.40 gprot/L) and 1×10^6 Jurkat cells (the concentration of protein is 0.39 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.