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CheKine™ Micro Mitochondrial Transhydrogenase-2 (TH-2) Activity Assay Kit

Cat #: KTB1840 Size: 48 T/96 T

	Micro Mitochondrial Transhydrogenase-2 (TH-2) Activity Assay Kit		
REF	**Cat #:** KTB1840	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Transhydrogenase (TH) is located in the inner membrane of mitochondria, also as known as Mitochondrial Respiratory Chain Complex VI. The enzyme catalyzes the mutual conversion of NADH+$NADP^+$ and NAD^++NADPH, and regulate the balance between NAD(H) and NADP(H) in mitochondria. The reverse reaction is called TH-2, which catalyzes NADPH and NAD^+ to generate $NADP^+$ and NADH. CheKine™ Micro Mitochondrial Transhydrogenase-2 (TH-2) Activity Assay Kit can be used to detect biological samples such as animal and plant tissues. Both NADH and NADPH have characteristic absorption peak at 340 nm, therefore, the hydrogen transfer reaction catalyzed by TH cannot has a absorbance change at 340 nm. Replaces NAD^+ with 3-acetylpyridyl adenine dinucleotide ($APAD^+$, synthetic substrate), TH-2 can catalyzes $APAD^+$ reduction to APADH and the reaction product has a characteristic absorption peak at 375 nm. In this kit, the TH-2 activity is quantified by measuring the rate of increase in light absorption at 375 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	50 mL	100 mL	-20℃
Reagent Ⅱ	25 mL	50 mL	-20℃
Reagent Ⅲ	9 mL	18 mL	4℃
Reagent Ⅳ	1	1	-20℃, protected from light
Reagent Ⅴ	1	1	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 375 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water

• Homogenizer or mortar (for tissue samples)

Reagent Preparation

Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃.

Reagent II: Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃.

Working Reagent: Prepared before use. Transfer Reagent Ⅳ and Ⅴ into Reagent Ⅲ and dissolve them and put them in 37℃ (mammal) or 25℃ (other species) water bath for 5 min. After the reagents were sub-packaged and stored away from light at -20℃ for 6 months, repeated freezing and thawing were prohibited.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Separation of cytoplasmic and mitochondrial proteins from tissues:Weigh 0.1 g tissue, add 1 mL Reagent Ⅰ and homogenize on ice;

2. Centrifuge at 600 g for 5 min at 4℃. Abandon precipitation, and transfer the supernatant to another centrifugal tube;

3. Centrifuge at 11,000 g for 5 min at 4℃.The supernatant in this step is to remove the cytoplasmic protein of mitochondria, which can be used to determine the TH-2 leakage from mitochondria (optional);

4. In step 3, the precipitation is mitochondria, adding 500 μL Reagent Ⅱ, ultrasonic fragmentation (ice bath, power 20% or 200 W, ultrasound 3 s, interval 10 s, repetition 30 times) for TH-2 activity determination.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 375 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Test Well (μL)
Sample	20
Working Reagent	180

3. Mix thoroughly, detect the absorbance at 375 nm as A1 immediately and A2 after 10 min. Finally calculate ΔA=A2-A1.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.25, increase the sample quantity appropriately. If ΔA is greater than 1.2, the sample can be appropriately diluted with Reagent Ⅰ or Reagent Ⅱ, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Calculation of the TH-2 activity

Calculated by fresh weight of samples

Unit definition: The production of 1 nmol APADH per min in g tissue is defined as a unit of enzyme activity.

TH-2 (U/g fresh weight)=$[\Delta A\times V_{Reaction\ volume}\div(\varepsilon\times d)\times 10^{9}]\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**$=149\times\Delta A\div W$**

$V_{Reaction\ volume}$: Total volume of reaction, 2×10^{-4} L; ε: The molar extinction coefficient of APADH, 6.7×10^{3} L/mol/cm; d: Light path of the 96-well, 0.5 cm; V_{Sample}: Added sample volume to the reaction system, 0.02 mL; $V_{Total\ sample}$: Added the Reagent Ⅱ volume, 0.5 mL; W: Sample weight, g; T: Reaction time, 10 min.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

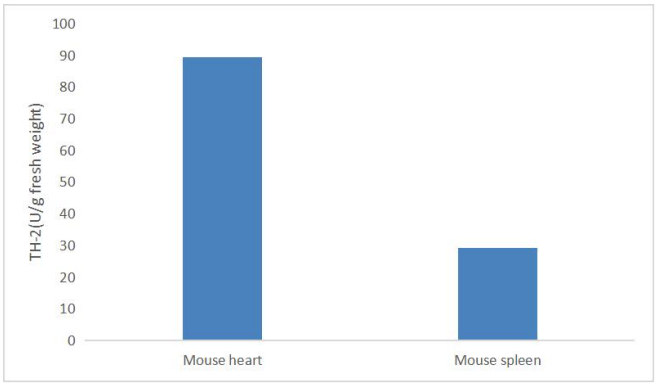

Figure 1. Determination of TH-2 activity in mouse heart and mouse spleen by this kit.

Recommended Products

Catalog No.	Product Name
KTB1850	CheKine™ Micro Mitochondrial complex Ⅰ Activity Assay Kit
KTB1860	CheKine™ Micro Mitochondrial complex Ⅱ Activity Assay Kit
KTB1870	CheKine™ Micro Mitochondrial complex Ⅲ Activity Assay Kit
KTB1880	CheKine™ Micro Mitochondrial complex Ⅳ Activity Assay Kit
KTB1890	CheKine™ Micro Mitochondrial complex Ⅴ Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.