Transhydrogenase-2 (TH-2) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: NA0395

Size:100T/48S

Components:

Extract solution: Liquid 100 mL×1. Storage at 4℃.

Reagent I: Powder×1. Storage at -20℃. Dissolve with 6 mL of Reagent III before use. It can be stored at -20℃ after repacking, but avoid repeated freezing and thawing.

Reagent II: Powder×1. Storage at 4℃. Dissolve with 10 mL of Reagent III before use.

Reagent III: Liquid 25 mL×1. Storage at 4℃.

Product Description:

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.

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.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, water bath, desk centrifuge, water bath, adjustable transferpettor, micro quartz cuvette/96 well plate (96 UV plate), mortar/homogenizer, ice and distilled water.

Procedure:

I. Extraction:

1) Collecting 0.1 g of tissue or 5 million cells, add 1 mL of extract solution, grinding on ice with mortar/homogenizer.

2) After centrifuge at 600 ×g for 10 minutes at 4℃.

3) Take the supernatant to other tube and centrifuge at 11000 ×g for 15 minutes at 4℃ to separate supernatant and sediment again.

4) The supernatant can used to detect TH-2 that leaking from mitochondria, which shows the effect of mitochondrial extraction.

5) Add 800 μL of Extraction solution to the sediment, splitting with ultrasonication (power 20%, work

time 5s, interval 10s, repeat 15 times), used to detect the enzyme activity of TH-2 and protein content.

II. Determination procedure:

1) Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 375 nm, set zero with distilled water.

2) Add reagents with the following list:

Reagent name (μL)	Test tube (A_T)	Contrast tube (Ac)
Reagent I	80	-
Reagent II	80	80
Sample	20	20
Reagent III	20	100

Add above reagents to a micro quartz cuvette/96 well UV plate in sequence, mix immediately. Start timing at the same time as adding the sample, record the initial absorbance A1 at 10 seconds under the 375 nm wavelength. Rapidly put cuvette and the reaction solution in 37℃(mammals) or 25℃ (other species) water-bath or incubator after colorimetry, react accurately for 3 minutes. Quickly take the cuvette and wipe dry it, colorimetry at 375 nm and record the absorbance A2 at 190 seconds, calculate $\Delta A_T = A_{T2} - A_{T1}$. $\Delta Ac = A_{C2} - A_{C1}$, $\Delta A = \Delta A_T - \Delta Ac$.

III. Calculation:

A. **micro quartz cuvette**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1nmol of APADH per minute every mg tissue protein.

TH-2 Activity (U/mg prot) $=[\Delta A \times Vrv \div (\varepsilon \times d)] \div (Cpr \times Vs) \div T = 498 \times \Delta A \div Cpr$

Vrv: Total reaction volume, 0.2 mL;

ε: The molar extinction coefficient of APADH, 6.7×10^{-3} mL/nmol/cm;

d: Light path of the cuvette, 1 cm;

Vs: Sample volume (mL), 0.02 mL;

Cpr: Sample protein concentration (mg/mL);

T: Reaction time (min), 3 minutes.

B. 96 well UV plate

The d-1cm in the formula can be replaced by d-0.6cm (96 well UV plate optical diameter).

Note:

1. Take two or three different samples for prediction before test. Dilute supernatant with Extraction solution or Reagent III if the $A_T > 1.5$ or $\Delta A > 0.25$, multiply dilute times in the formula.
2. The protein concentrate of the sample needs to be determined by yourself and our PC0020 BCA Protein Assay Kit is recommended. Since the extract contains a relatively high concentration of protein, it is necessary to subtract the protein content of the extract itself when determining the protein concentration of the sample.
3. ΔA_T or ΔAc negative values are normal.
4. **It is recommended to use the sample protein concentration to calculate the enzyme activity.** If the

sample fresh weight is used to calculate, the enzyme activity of cytoplasmic extract needs to be measured, and the sum of supernatant and precipitation enzyme activity is the total enzyme activity.

5. The reagent in this kit is enough to complete 100 tube reaction.
6. Calculate by sample weight as follows: (the number of samples tested is 100T/24S)

A. micro quartz cuvette

1) Supernatant:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1nmol of APADH per minute every gram tissue weight

TH-2 Activity(U/g)=[ΔA1×Vrv÷(ε×d)]÷(W÷Ve×Vs)÷T=498×ΔA1÷W

ΔA1: Supernatant absorbance;

Vrv: Total reaction volume, 0.2 mL;

ε: The molar extinction coefficient of APADH, 6.7×10^{-3} mL/nmol/cm;

d: Light path of the cuvette, 1 cm;

Ve: Extract solution volume, 1 mL;

Vs: Sample volume (mL), 0.02 mL;

W: Sample weight, g;

T: Reaction time (min), 3 minutes.

2) Sediment:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1nmol of APADH per minute every gram tissue weight

TH-2 Activity(U/g) =[ΔA2×Vrv÷(ε×d)]÷(W÷Ve×Vs)÷T=398×ΔA2÷W

Vrv: Total reaction volume, 0.2 mL;

ε: The molar extinction coefficient of APADH, 6.7×10^{-3} mL/nmol/cm;

d: Light path of the cuvette, 1 cm;

Ve: Extract solution volume, 0.8 mL;

Vs: Sample volume (mL), 0.02 mL;

W: Sample weight, g;

T: Reaction time (min), 3 minutes.

3) Total activity

Total activity is the sum of TH-2 activity in supernatant and sediment.

TH-2 (U/g) =498×ΔA1÷W+398×ΔA2÷W.

B. 96 well UV plate

The d-1cm in the formula can be replaced by d-0.6cm (96 well UV plate optical diameter).