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Thioredoxin Reductase (TrxR) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: AK0481

Size:100T/96S

Components:

Reagent I: 120 mL×1. Storage at 4℃ .

Reagent II: 3mL×1. Storage at 4℃ and protect from light. Dissolve with 5 mL of distilled water when the solution will be used.

Reagent III: Powder×2. Storage at 4℃ . protect from light. Dissolve with 1.667 mL of distilled water when the solution will be used.

Reagent IV: 30μL×1. Storage at -20℃ . Dilute Reagent IV with ethanol 10 times according to the number of samples before use.

Product Description:

TrxR is a NADPH-dependent dimer selenase and includes FAD structure domain. TrxR belongs to pyridine nucleotide-disulfide REDOX enzyme, and form thioredoxin system with thioredoxin and NADPH. The activity of TrxR is similar with GR. TrxR could catalyzes GSSG reduce to GSH, which is the key enzyme in glutathione REDOX cycle.

TrxR catalyzes NADPH to reduceDTNB form TNB and $NADP^+$, TNB has a absorbance at 412 nm, but reduced glutathione reacts with DTNB to form TNB, so the 2-Vinylpyridine in this kit can inhibit reduced glutathione in sample, the activity of TrxR can be calculated by detecting increase rate of TNB at 412 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, ultra-micro glass cuvette/96 well flat-bottom plate,water bath, low temperature centrifuge, mortar/homogenizer, adjustable pipette,distilled water.

Procedure

I. Sample preparation:

1. Tissue:

Add 1 mL of Reagent I into 0.1 g of tissue, fully grinding on ice. Centrifuge at 10000 rpm for 10 minutes at 4℃ and take the supernatant on ice for test. Before test, mix supernatant and Reagent IV at a ratio of 50:1(add 2 μL of Reagent IV to 100 μL of supernatant), water bath at 37℃ for 30 minutes, then keep on ice for test.

2. Bacteria/cell:

Suggested 5 million with 1 mL of Reagent I, Splitting bacteria and cell with ultrasonic(ice bath,

power 300W, work time 3 s, interval 7 s, for 3 minutes), centrifuge at 10000 rpm and 4℃ for 10 minutes. The supernatant on ice is used for test. before test, mix supernatant and Reagent IV at a ratio of 50:1(add 2 μL of Reagent IV to 100 μL of supernatant), water bath at 37℃ for 30 minutes, then keep on ice for test.

III. Procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 412 nm, set the zero with distilled water.
2. Preheat reagent1 at 37℃(mammal), 25℃(other) in water bath for 30 minutes.
3. Blank tube: take a micro glass cuvette/96 well flat-bottom plate, add 20 μLof Reagent II, 20 μLof Reagent III, 160 μLof Reagent I, mix them quickly, and then measure the absorbance at 412nm for 10s. Take out the absorbance at 412 nm in a 37℃ water bath for 5min and record it as A1 and A2. Calculate $\triangle A_B$=A2-A1.
4. Measuring tube: take a micro glass cuvette/96 well flat-bottom plate, add 20 μLof Reagent II, 20 μLof Reagent III, 140 μLof Reagent I, 20 μLof supernatant, mix it quickly, and then measure the absorbance at 412nm for 10s, take out the absorbance at 412 nm quickly in a 37℃ water bath for 5min, and record it as A3 and A4. $\triangle A_T$= A4-A3.

III. Calculation:

A. micro glass cuvette:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymecatalyzes the production of1μmol ofTNB at 37℃(mammal), 25℃(other) per minuteevery mg of protein.

TrxR(U/mgprot)=[ΔA(T)-ΔA(B)]÷(ε×d)×109×Vrv÷(Vs×Cpr)÷T =147×[ΔA(T)-ΔA(B)]÷Cpr

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymecatalyzes the production of1μmol ofTNB at 37℃(mammal), 25℃(other) per minuteevery gram of sample.

TrxR(U/g)=[ΔA(T)-ΔA(B)]÷(ε×d)×10^9 ×Vrv÷(Vs÷Vsv×W)÷T=147×[ΔA(T)-ΔA(B)]÷W

3. Cells:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of1μmol ofTNB at 37℃(mammal), 25℃(other) per minute every 10^4 cell.

TrxR(U/10^4cell)=[ΔA(T)-ΔA(B)]÷(ε×d)×10^9 ×Vrv÷(N÷Vsv×Vs)÷T=147×[ΔA(T)-ΔA(B)]÷N

ε: TNB molar extinction coefficient， 1.36×10^4 L/mol/cm;

d: Light path of cuvette, 1cm;

Vrv: Total reaction volume, 200 μL=2×10^{-4} L;

Vs: Supernatant volume (mL), 0.02 mL;

Cpr: Sample protein concentration (mg/mL); need to detect separately, suggest use PC0020, BCA Protein Assay Kit;

T: Reaction time (min), 5 minutes;

W: Sample weight(g);

Vsv: Extract solution volume, 1 mL;

N: Amount of cells,10^4.

B. 96 well flat-bottom plate

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymecatalyzes the production of1μmol ofTNB at 37℃(mammal), 25℃(other) per minute every mg of protein.

TrxR (U/mg prot) =[ΔA(T)-ΔA(B)]÷(ε×d)×10^9×Vrv÷(Vs×Cpr)÷T

= 245×[ΔA(T)-ΔA(B)]÷Cpr

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymecatalyzes the production of1μmol ofTNB at 37℃(mammal), 25℃(other) per minute every gram of sample.

TrxR(U/g) = [ΔA(T)-ΔA(B)]÷(ε×d)×10^9×Vrv÷(Vs÷Vsv×W)÷T

= 245×[ΔA(T)-ΔA(B)]÷W

3. Cells:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of1μmol ofTNB at 37℃(mammal), 25℃(other) per minute every 10^4 cell.

TrxR(U/10^4cell) = [ΔA(T)-ΔA(B)]÷(ε×d)×10^9×Vrv÷(N÷Vsv×Vs)÷T

= 245× [ΔA(T)-ΔA(B)]÷N

ε: TNB molar extinction coefficient at 412 nm,1.36×10^4 L/mol/cm;

d: Light path of cuvette, 0.6 cm;

Vrv: Total reaction volume,200 μL=2×10^{-4} L;

Vs: Supernatant volume (mL), 0.02 mL;

Cpr: Sample protein concentration (mg/mL); need to detect separately, suggest use PC0020, BCA Protein Assay Kit;

T: Reaction time (min), 5 minutes;

W: Sample weight(g);

Vsv: Extraction volume, 1 mL;

N: Amount of cells,10^4.

Note:

1. Dilute 5 times with distilled water when detecting mammalian tissue and blood samples, detect quickly as soon as possible.

2. Because the extract solution contains a certain concentration of protein (about 0.1 mg/mL), the protein content of the extract solution itself needs to be subtracted when determining the protein concentration of the sample.

Experimental instances：

1. Take 0. 1g of Chinese rose petals, add 1mL of extract solution, fully grinding on ice. Centrifuge at 10000 rpm for 10 minutes at 4℃, take the supernatant and place it on ice for test according to the measured steps. Calculate ΔA_T=A4-A3=0.8600-0.8177=0.0423， ΔA_B=A2-A1=0.0792-0.0727=0.0065,

calculate the enzyme activity according to sample weight:

TrxR（U/g weight）=147×（ΔA_T-ΔA_B）÷W=5.26 U/g weight.

2. Take 0. 1g of liver, add 1mL of extract solution, fully grinding on ice. Centrifuge at 10000 rpm for 10 minutes at 4℃, take the supernatant and place it on ice for test according to the measured steps. CalculateΔA_T=A4-A3=0.8538-0.2102=0.6436， ΔA_B=A2-A1=0.0792-0.0727=0.0065, calculate the enzyme activity according to sample weight:

TrxR（U/g weight）=147×（ΔA_T-ΔA_B）÷W×4（dilution ridio）=3746. 148 U/g weight.

Recent Product citations

[1] Li B, Li D, Jing W, et al. Biogenic selenium and its hepatoprotective activity[J]. Scientific reports, 2017, 7(1): 1- 11.

[2] Zhang L, Fan J, He J, et al. Regulation of ROS–NF-κB axis by tuna backbone derived peptide ameliorates inflammation in necrotizing enterocolitis[J]. Journal of cellular physiology, 2019, 234(8): 14330- 14338.

Related products：

AK0478/ AK0477	Reduced Glutathione（GSH）Assay Kit
AK0476/ AK0475	Oxidized Glutathione（GSSG）Assay Kit
AK0474/ AK0473	Glutathione Peroxidase（GPX）Assay Kit
AK0558/ AK0557	Glutathione S-transferase (GST) Activity Assay Kit