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CheKine™ Micro NADH Oxidase (NOX) Activity Assay Kit

Cat #: KTB1021 Size: 48 T/96 T

	Micro NADH Oxidase (NOX) Activity Assay Kit		
REF	**Cat #:** KTB1021	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells, Bacteria		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

NADH Oxidase (NOX, EC 1.6.99.3) is widely found in animals, plants, microorganisms and cultured cells, and can directly oxidize NADH to NAD in the presence of oxygen. This enzyme is not only involved in the regeneration of NAD, but also closely related to the immune response. CheKine™ Micro NADH Oxidase (NOX) Activity Assay Kit provides a simple method for detecting NOX activity in a variety of biological samples such as animal and plant tissues, cells and bacteria. In the assay, NOX can oxidize NADH to NAD, the oxidation of NADH is coupled with the reduction of 2,6 dichlorophenol indigo (DCPIP), the blue DCPIP is reduced to colorless DCPIP, and the reduction rate of blue DCPIP is measured at 600 nm which can reflect NADH oxidase activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Sample Lysis Buffer	50 mL	100 mL	-20℃
Mitochondria Lysis Buffer	12 mL	24 mL	4℃
Protease Inhibitor	0.75 mL	1.5 mL	-20℃
Chromogen	12 mL	24 mL	4℃, protected from light
Substrate	1	1	-20℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 600 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Freezing centrifuge, incubator
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Sample Lysis Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Mitochondria Lysis Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Protease Inhibitor: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Chromogen: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Working Substrate: Add 5 mL deionized water to each Substrate before use, and store the reagent at -20℃, place on ice to be used, avoid repeated freezing and thawing.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.

Separation of cytoplasmic proteins and mitochondrial proteins in tissues, bacteria or cells:

1. Accurately weigh 0.1 g tissue or collect 5×10^6 cells or bacteria, add 1 mL Sample Lysis Buffer and 10 μL Protease Inhibitor, and homogenize on ice with dounce homogenizer.
2. Centrifuge at 4℃, 600 g for 5 min.
3. Transfer the supernatant to another centrifuge tube, centrifuge at 4℃, 11,000 g for 10 min.
4. The supernatant is the cytoplasmic protein which removes mitochondria and can be used to determine the NOX leakage from the mitochondria (this step is optional). The second time precipitation is mitochondria, 200 μL Mitochondria Lysis Buffer and 2 μL Protease Inhibitor are added, ultrasonic 5 min (power 20%, ultrasound 3 s, interval 7 s, repeated 30 times), used for NOX activity determination.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 600 nm, visible spectrophotometer was returned to zero with deionized water.
2. Put Chromogen in a 25℃ or 37℃ (mammal) Incubator for 30 min.
3. Add the following reagents to the 96-well plate or microglass cuvette:

Reagent	Blank Well (μL)	Test Well (μL)
Sample	0	10
Sample Lysis Buffer	50	0
Chromogen	200	200
Working Substrate	0	40
Mix well, record the 10 s absorbance value at 600 nm as A_1, and incubate at 25℃ or 37℃ (Mammals) In the incubator, after 1 min, quickly take out and record 1 min 10 s absorbance value as A_2.		

4. Calculate $\Delta A = A_1-A_2$. Record ΔA_{Blank}, ΔA_{Test}, $\Delta\Delta A = \Delta_{Test}-\Delta A_{Blank}$.

Note: Blank well only need to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. The optimal reaction temperature of NOX in different samples is slightly different, and it can be adjusted between 25-37℃. Since the enzyme activity is calculated based on the reaction rate, when using a 96-well plate, please control the number of samples to be measured at one time according to the operating speed.

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.

A. 96-well plates calculation formula

1. Calculated by sample fresh weight

Active unit definition: A 0.005 change of A600 value per g of sample per min was catalyzed at reaction system is defined as a unit of enzyme activity.

NOX supernatant (U/g)=$\Delta\Delta A_{Supernatant}$÷0.005×$V_{Total}$÷(W÷$V_{Extrac\ t}$×$V_{Sample}$)÷T=**5,050×$\Delta\Delta A_{Supernatant}$÷W**

NOX precipitation (U/g)=$\Delta\Delta A_{Precipitation}$÷0.005×$V_{Total}$÷(W÷$V_{Total\ Sample}$×$V_{Sample}$)÷T=**1,010×$\Delta\Delta A_{Precipitation}$÷W**

NOX total (U/g)=$NOX_{Supernatant}$+$NOX_{Precipitation}$=**5,050×$\Delta\Delta A_{Supernatant}$÷W+1,010×$\Delta\Delta A_{Precipitation}$÷W**

2. Calculated by bacteria or cells number

Active unit definition: A 0.005 change of A600 value per 10^4 bacteria or cells per min was catalyzed at reaction system is defined as a unit of enzyme activity.

NOX ($U/10^4$)=ΔΔA÷0.005×V_{Total}÷(V_{Sample}÷$V_{Total\ Sample}$×500)÷T=**2.02×ΔΔA**

B. Microglass cuvette calculation formula

1. Calculated by sample fresh weight

Active unit definition: A 0.01 change of A600 value per g of sample per min was catalyzed at reaction system is defined as a unit of enzyme activity.

NOX supernatant (U/g)=$\Delta\Delta A_{Supernatant}$÷0.01×$V_{Total}$÷(W÷$V_{Extrac\ t}$×$V_{Sample}$)÷T=**2,525×$\Delta\Delta A_{Supernatant}$÷W**

NOX precipitation (U/g)=$\Delta\Delta A_{Precipitation}$÷0.01×$V_{Total}$÷(W÷$V_{Total\ Sample}$×$V_{Sample}$)÷T=**505×$\Delta\Delta A_{Precipitation}$÷W**

NOX total (U/g)=$NOX_{Supernatant}$+$NOX_{Precipitation}$=**2,525×$\Delta\Delta A_{Supernatant}$÷W+505×$\Delta\Delta A_{Precipitation}$÷W**

2. Calculated by bacteria or cells number

Active unit definition: A 0.01 change of A600 value per 10^4 cells per min was catalyzed at reaction system is defined as a unit of enzyme activity.

NOX ($U/10^4$)=ΔΔA÷0.01×V_{Total}÷(V_{Sample}÷$V_{Total\ Sample}$×500)÷T=**1.01×ΔΔA**

Where: W: Sample fresh weight, g; T: reaction time, 1 min; V_{Total}: Total reaction volume, 0.25 mL; V_{Sample}: Sample volume added, 0.01 mL; $V_{Extract}$: Extract solution volume added, 1.01 mL; $V_{Total\ Sample}$: precipitation resuspension volume, 0.202 mL; Cpr: Sample protein concentration, mg/mL; 500: total number of cells or bacteria, 5×10^6.

Note: If the sample fresh weight is used, it needs to detect the cytoplasmic extract enzyme activity as well. The sum of the supernatant and precipitation enzyme activity is the total enzyme activity.

Recommended Products

Catalog No.	Product Name
KTB1020	CheKine™ Micro Coenzyme Ⅰ NAD(H) Assay Kit
KTB1010	CheKine™ Micro Coenzyme Ⅱ NADP(H) Assay Kit
KTB1041	CheKine™ Micro Hydrogen Peroxide (H_2O_2) Assay Kit

Disclaimer

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