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CheKine™ Micro Non-protein Sulfhydryl Content Assay Kit

Cat #: KTB1551 Size: 96 T

	Micro Non-protein Sulfhydryl Content Assay Kit		
REF	**Cat #:** KTB1551	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

The sulfhydryl group in organism mainly includes non-protein sulfhydryl group and protein sulfhydryl group. Sulfhydryl compounds have important detoxification function in vivo. It has very important physiological significance to the self-regulation of organism. CheKine™ Micro Non-protein sulfhydryl Content Assay Kit can detect samples such as animal and plant tissues, plasma, serum or other liquid samples.The principle is that the thiol group reacts with 5,5 '- dithio-bis-nitrobenzoic acid (DTNB) to form a yellow compound. It has a maximum absorption peak at 412 nm.

Materials Supplied and Storage Conditions

Kit components	Size (96 T)	储存条件
Extraction Buffer	100 mL	4℃
Reagent Ⅰ	18 mL	4℃
Reagent Ⅱ	1	4℃, protected from light
Standard	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 412 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Ice maker, freezing centrifuge
- Deionized water, anhydrous ethanol
- Homogenizer (for tissue samples)

Reagent Preparation

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

Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent II: Prepared before use; Add 1 mL anhydrous ethanol and dissolve thoroughly for later use; Store at 4℃, protected from

light.

Standard: Prepared before use; Add 1.65 mL of Extraction Buffer to dissolve it into 50 μmol/mL standard solution, which could be stored for 1 month in the dark at 4℃.

Standard preparation: Using 50 μmol/mL Cysteine Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Num.	Standard volume	Deionized water volume (μL)	Concentration (μmol/mL)
Std.1	32 μL 50 μmol/mL Standard	968	1.6
Std.2	500 μL of Std.1 (1.6 μmol/mL)	500	0.8
Std.3	500 μL of Std.2 (0.8 μmol/mL)	500	0.4
Std.4	500 μL of Std.3 (0.4 μmol/mL)	500	0.2
Std.5	500 μL of Std.4 (0.2 μmol/mL)	500	0.1
Std.6	500 μL of Std.5 (0.1 μmol/mL)	500	0.05
Std.7	500 μL of Std.6 (0.05 μmol/mL)	500	0.025
Std.8	500 μL of Std.7 (0.025 μmol/mL)	500	0.0125
Std.9	500 μL of Std.8 (0.0125 μmol/mL)	500	0.0063
Blank	0	500	0

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

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 4h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2. Plasma, Serum or other Liquid samples: Add 1 mL of Extract solution to 0.1 mL of serum (plasma) or culture medium. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 412 nm. Visible spectrophotometer was returned to zero with deionized water.

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

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample supernatant	0	0	60
Standard	0	60	0
Reagent Ⅰ	130	130	130
Reagent Ⅱ	0	20	20
Deionized Water	80	0	0

3. Mix well, Incubate for 30 min at 25℃. The absorbance value is measured at 412 nm. The blank well is recorded as A_{Blank}, the standard well is marked as $A_{Standard}$, and the test well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$,

$\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Standard curves and blank holes only need to be tested 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 2.0, the sample can be appropriately diluted with Extraction Buffer, 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.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation y=kx+b.

2. Calculation of the non-protein sulfhydryl content

(1) Calculated by sample protein concentration

Non-protein sulfhydryl content (μmol/mg prot)=$x\times V_{Sample\ total}\div Cpr$**=x÷Cpr**

(2) Calculated by fresh weight of samples

Non-protein sulfhydryl content (μmol/g fresh weight)=$x\times V_{Sample\ total}\div W$**=x÷W**

(3) Calculated by volume of liquid samples

Non-protein sulfhydryl content (μmol/L)**=x×5**

$V_{Sample\ total}$: add Extraction Buffer volume to sample, 1 mL; Cpr: sample protein concentration, mg/mL; W: weight of sample, g; 5: the sample dilution factor of serum and other liquid samples.

Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

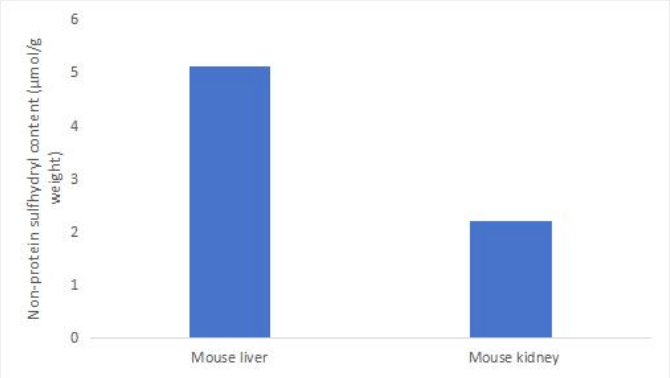

Figure 1. Determination of non-protein sulfhydryl groups in mouse liver and kidney by this kit

Recommended Products

Catalog No.	Product Name
KTB1500	CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit
KTB1510	CheKine™ Micro Uric Acid (UA) Assay Kit
KTB1550	CheKine™ Micro Ceruloplasmin Activity Assay Kit

Disclaimer

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