CheKine™ Micro Glutathione Reductases (GR) Activity Assay Kit

Cat #: KTB1620 Size: 96 T/96 T×5

	Micro Glutathione Reductases (GR) Activity Assay Kit		
REF	**Cat #:** KTB1620	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal Tissues, Cells, Bacteria		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Glutathione Reductases (GR) is a flavin protein redox enzyme widely present in eukaryotes and prokaryotes. GR catalyzes the reduction of GSSG to GSH. It is one of the key enzymes in the glutathione redox cycle (generally GR is replaced by Thioredoxin Reductase (TrxR) in insects). GR catalyzes the reduction of GSSG by NADPH to GSH, which helps maintain the GSH/GSSG ratio in the body. GR plays a key role in scavenging reactive oxygen species in response to oxidative stress, and GR is also involved in the ascorbate-glutathione cycle pathway. CheKine™ Micro Glutathione Reductases (GR) Activity Assay Kit provides a simple, convenient and rapid method for the detection of GR content, which is suitable for serum, plasma, animal tissues, cells, bacteria, etc. sample. The principle is that GR can catalyze the reduction of NADPH to GSSG to regenerate GSH, and NADPH dehydrogenates to produce $NADP^+$; NADPH has a characteristic absorption peak at 340 nm, while $NADP^+$ has no absorption peak at this wavelength; NADPH dehydrogenation rate is determined by measuring the decrease rate of absorbance at 340 nm to calculate GR activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	96 T×5	
Assay Buffer	100 mL	100 mL×5	4℃
Substrate	2 mL	2 mL×5	4℃, protected from light
GR Cofactor	1	1×5	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Freezing centrifuge, water bath
- Deionized water
- Dounce homogenizer (for tissue samples)

Reagent Preparation

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

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

GR Cofactor: Powder. Add 2 mL of deionized water to each tube before use, mix well, prepare it for use, and placed it on ice. Store at -20℃, protect from light.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month. The whole processes need to be carried out on ice, and the enzyme activity should be determined on the same day, to avoid repeated freeze-thaw of the homogenate solution.

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

2. Bacteria or Cells: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL cold Assay Buffer to ultrasonically disrupt the cells or bacteria 5 min on ice (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

3. Serum or Plasma: Tested directly.

Note: In the detection of GR activity in Cells, the Cells number must be between 3-5x10^6, and the extraction of GR in cells can be followed by Assay Buffer grinding or ultrasonic treatment, cells cannot be treated by lysate. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Put Assay Buffer in a 25℃ (for other species) or 37℃ (for mammals) Incubator for 30 min.

3. Add the following reagents to the 96-well UV plate or microquartz cuvette:

Reagent	Blank Well (μL)	Test Well (μL)
Sample	0	20
Assay Buffer	170	150
Substrate	10	10
GR Cofactor	20	20
Mix well, the absorbance values were measured at 340 nm for 10 s and 190 s. The blank well marked as A_1 and A_2, and the test well marked as A_3 and A_4. $\Delta A_{Blank}=A_1-A_2$, $\Delta A_{Test}=A_3-A_4$.		

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 to ensure that the change in absorbance within 180s is linear. Generally, mammalian tissue must be diluted 2 to 5 times with Assay Buffer. Since the enzyme activity is calculated based on the reaction rate, when using a 96-well UV 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 UV plates calculation formula as below:

(1) By protein concentration

Active unit definition: at 25℃ or 37℃, 1 μmol NADPH oxidation per milligram of protein per min was catalyzed at pH 8.0.

GR activity (U/mg prot)=$[(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times V_{Total}\times 10^{6}]\div(Cpr\times V_{Sample})\div T$ **=1.072×($\Delta A_{Test}-\Delta A_{Blank}$)÷Cpr**

(2) By sample fresh weight

Active unit definition: at 25℃ or 37℃, 1 μmol NADPH oxidation per gram of sample per min was catalyzed at pH 8.0.

GR activity (U/g)=$[(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times V_{Total}\times 10^{6}]\div(V_{Sample}\div V_{Sample\ Total}\times W)\div T$ **=1.072×($\Delta A_{Test}-\Delta A_{Blank}$)÷W**

(3) By cells number or bacteria

Active unit definition: at 25℃ or 37℃, 1 μmol NADPH oxidation per 10^4 cells of sample per min was catalyzed at pH 8.0.

GR activity (U/10^4)=$[(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times V_{Total}\times 10^{6}]\div(500\times V_{Sample}\div V_{Sample\ Total})\div T$ **=0.0021×($\Delta A_{Test}-\Delta A_{Blank}$)**

(4) By liquid volume

Active unit definition: at 25℃ or 37℃, 1 μmol NADPH oxidation per mL of liquid per min was catalyzed at pH 8.0.

GR activity (U/mL)=$[(\Delta A_{Test}-\Delta A_{Blank})\div(\varepsilon\times d)\times V_{Total}\times 10^{6}]\div V_{Sample}\div T$ **=1.072×($\Delta A_{Test}-\Delta A_{Blank}$)**

Where: $\Delta A_{Blank}=A_1-A_2$, $\Delta A_{Test}=A_3-A_4$; ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV Plate light path, 0.5 cm; V_{Total}: total volume of reaction system, 200 μL= 2×10^{-4} L; 10^6:1 mol=1×10^6 μmol; Cpr: protein concentration of supernatant, mg/mL; W: sample mass, g; V_{Sample}: volume of supernatant added to the reaction system, 20 μL= 2×10^{-2} mL; $V_{Sample\ Total}$: volume of extraction solution, 1 mL; T: reaction time, 3 min; 500: number of cells or bacteria, 5×10^6.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Recommended Products

Catalog No.	Product Name
KTB1600	CheKine™ Micro Reduced Glutathione (GSH) Assay Kit
KTB1610	CheKine™ Micro Glutathione Oxidized (GSSG) Assay Kit
KTB1630	CheKine™ Micro Glutathione S-Transferase (GST) Assay Kit
KTB1640	CheKine™ Micro Glutathione Peroxidase (GSH-Px) Assay Kit
KTB1650	CheKine™ Micro Thioredoxin Reductase (TrxR) Assay Kit
KTB1660	CheKine™ Micro Thioredoxin Peroxidase (TPX) Assay Kit

Disclaimer

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