

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Glutathione Reductase Activation Coefficient (GRAC) Assay Kit

Catalog No.: BC00165

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale)	order@enkilife.com
✉ Email (Techsupport)	techsupport@enkilife.com
☎ Tel:	0086-27-87002838
🌐 Website:	www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Glutathione Reductase Activation Coefficient (GRAC) Assay Kit
Detection Method	Colorimetric
Assay Type	Quantitative
Sample Type	Tissue, serum, plasma
Detection Instrument	Spectrophotometer (420 nm)

Product Introduction

In riboflavin deficiency, the glutathione reductase activation coefficient rises rapidly, yet it returns to normal after riboflavin supplementation. Therefore, the Glutathione Reductase Activation Coefficient (GRAC) serves as an accurate biomarker for evaluating the overall riboflavin status in the body during chronic riboflavin deficiency. Assessing riboflavin nutritional status based on GRAC values boasts advantages including high sensitivity, good stability, high accuracy, microscale sample requirement, and the capability to reflect riboflavin metabolism and utilization in vivo.

Principle

Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH). Flavin adenine dinucleotide (FAD) acts as the prosthetic group of this enzyme. Accordingly, insufficient FAD caused by riboflavin deficiency will reduce the activity of GR. The nutritional status of riboflavin in the body can be evaluated by measuring the glutathione reductase activation coefficient.

Components

No.	Components	Size	Notes
Reagent 1	Buffer	32 mL	Store at 4°C
Reagent 2	Substrate stock solution	0.22 mL	Store at -20°C protected from light.
	Substrate diluent	2.2 mL	Store at -20°C

Reagent 3	Matrix powder	2 vials	Store at -20°C protected from light.
	Matrix diluent	2.5 mL×2	Store at -20°C
Reagent 4	Accelerator powder	2 vials	Store at -20°C protected from light.
	Accelerator diluent	1.3 mL×2	Store at -20°C
Reagent 5	Precipitant	40 mL×2	Store at 4°C
Reagent 6	Chromogenic reagent powder	2 vials	Store at 4°C protected from light.
	Chromogenic reagent diluent	50 mL×2	Store at 4°C protected from light.

Preparation of **Reagent 2** Working Solution: Before use, dilute the substrate stock solution 10-fold at a volume ratio of substrate stock solution to substrate diluent = 1:9 to prepare the substrate working solution. Any unused working solution can be stored at 4°C within three days; store at -20°C if held for more than three days.

Preparation of **Reagent 3** Working Solution: Before use, fully dissolve one vial of matrix powder into one bottle of matrix diluent to prepare the matrix working solution. Unused working solution can be stored at 4°C within three days; store at -20°C if kept for more than three days.

Preparation of **Reagent 4** Working Solution: Before use, fully dissolve one vial of accelerator powder into one bottle of accelerator diluent to prepare the accelerator working solution. Unused working solution can be stored at 4°C within three days; store at -20°C if kept for more than three days.

Preparation of **Reagent 6** Working Solution: Before use, fully dissolve one vial of chromogenic reagent powder into one bottle of chromogenic reagent diluent to prepare the chromogenic working solution. Store at 4°C protected from light.

Storage

The kit has a validity period of 6 months.

Preparation

Visible spectrophotometer with a detection wavelength of 420 nm, 0.5 cm path length cuvettes, 37 °C water bath or incubator, boiling water bath, tabletop low-speed centrifuge, pipettes of various specifications, double-distilled water, normal saline (0.9%), vortex mixer, test tubes or centrifuge tubes, protein assay reagents (available from our company).

Operation process

	Blank tube	Assay tube	Control tube
Distilled water (μL)	270		
Samples (μL)		300	300
Reagent 1 (μL)	300	250	300
Reagent 2 Working Solution (μL)		20	20
Reagent 3 Working Solution (μL)	50	50	50
Reagent 4 Working Solution (μL)	50	50	
Incubate at 37 °C for 30 minutes.			
Reagent 5 (μL)	800	800	800
Mix thoroughly, centrifuge at 4000 rpm for 10 min, collect the supernatant for subsequent experiments.			
Supernatant (μL)	300	300	300
Reagent 6 Working Solution (μL)	1000	1000	1000
Mix well and stand for 5 minutes. Zero the instrument with double-distilled water using a cuvette with 0.5 cm optical path at a wavelength of 420 nm, then measure the absorbance (A) of each tube.			

Calculation

$$\text{GRAC} = \frac{A_{\text{Assay}} - A_{\text{Blank}}}{A_{\text{Control}} - A_{\text{Blank}}}$$

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.

3. Only 1 to 2 blank tubes are required.