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UC Davis - Glutathione Reductase 🔗

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May 14, 2019

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ABSTRACT

Summary:

Glutathione reductase (GR, EC 1.6.4.2) is a flavoprotein that catalyzes the NADPH- dependent reduction of oxidized glutathione (GSSG) to glutathione (GSH). This enzyme is essential for the GSH redox cycle which maintains adequate levels of reduced cellular GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress. The Cayman Chemical Glutathione Reductase Assay Kit measures GR activity by measuring the rate of NADPH oxidation. The oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm. Since GR is present at rate limiting concentrations, the rate of decrease in the A340 is directly proportional to the GR activity in the sample. The Cayman GR Assay Kit is provided in a convenient 96 well plate format and can be used to measure GR activity inplasma, erythrocyte lysates, tissue homogenates, and cell lysates.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=127&docType=Protocol

MATERIALS

NAME ⌄	CATALOG # ⌄	VENDOR ⌄	CAS NUMBER ⌄	RRID ⌄
Assay Kit	703202	Cayman Chemical Company		
Buffer				
Standard				
GSSG				
NADPH				

MATERIALS TEXT

Note:
Cayman Chemical RRID:SCR_008945

1 Background or Non-enzymatic Wells - add 120 µl of Assay Buffer and 20 µl ofGSSG to three wells.

2 Positive Control Wells (Baker's yeast GR) - add 100 µl of Assay Buffer, 20 µl of GSSG, and 20 µl of dilmed GR (comrol) to three wells.

3 Sample Wells - add 100 µl of Assay Buffer, 20 µl of GSSG, and 20 µl of sample to three wells. To obtain reproducible results, the amount ofGR added to the well should cause an absorbance decrease bctvveen 0.008 and O.I/min. When necessary, samples should be diluted with Sample Buffer or concentrated with an Amicon centrifuge concentrator with a molecular weight cut-off of 10,000 to bring the enzymatic activity to this level. ***NOTE:*** *The amount of sample added to the well should always be 20 µl. To determine if an additional sample control*

should be performed, see the Interferences section. (below)/(page 14)

Interferences

• *Samples that have a high intrinsic absorbance at 340 nm may exceed the absorbance maximum of the plate reader. Therefore, samples with an initial absorbance > 1.2 should be diluted with Sample Buffer until the absorbance is lowered. For example, hemoglobin absorbs significantly at 340 nm, and thus erythrocyte lysates must be diluted before assaying.*

• *Samples containing high levels of GSSG or NADPH consuming enzymes will cause the GR levels to be overestimated. A blank without GSSG should be performed to assess nonspecific oxidation of NADPH. GSSG can be removed from the sample by either dialysis or passing through a gel filtration column.*

4   Initiate the reactions by adding 50µl of NADPH to all the wells you are using. Make sure to note the precise time you started and add the NADPH as quickly as possible.

5   Carefully shake the 96-well plate for a few seconds to mix.

6   Read the absorbance once every minute at 340 nm using a plate reader to obtain at least 5 time points. **NOTE**: *The initial absorbance of the sample wells should not be above 1.2 or below 0.5.*

7   **Calculations**

1. Determine the change in absorbance (ΔA_{340}) per minute by:

   a. Plotting the absorbance values as a function of time to obtain the slope (rate) of the linear portion of the curve (a graph is shown on page 12 using Baker's yeast GR)

OR

   b. Select two points on the linear portion of the curve and determine the change in absorbance during that time using the following equation:

$$\Delta A_{340}/min. = \frac{*|\,A_{340}\,(Time\ 2) - A_{340}\,(Time\ 1)\,|}{Time\ 2\ (min.) - Time\ 1\ (min.)}$$

*Use the absolute value.

2. Determine the rate of ΔA_{340}/min. for the background or non-enzymatic wells and subtract this rate from that of the sample wells.

3. Use the following formula to calculate the GR activity. The reaction rate at 340nm can be determined using the NADPH extinction coefficient of $0.00373 \mu M^{-1}$*. One unit is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of NADPH to NADP^{+} per minute at 25ºC.

$$GR\ Activity = \frac{\Delta A_{340}/min.}{0.00373\ \mu M^{-1}} \times \frac{0.19\ ml}{0.02\ ml} \times Sample\ dilution = nmol/min/ml$$

*The actual extinction coefficient for NADPH at 340 nm is $0.00622\ \mu M^{-1} cm^{-1}$. This value has been adjusted for the pathlength of the solution in the well (0.6cm).