Glutathione Reductase Protocol

Version: 1
Replaced by version
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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.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Assay Kit	Cayman	703202
Buffer		
Standard		
GSSG		
NADPH		

Protocol:

1. **Background or Non-enzymatic Wells** - add 120 µl of Assay Buffer and 20 µl of GSSG 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 diluted GR (control) 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 of GR added to the well should cause an absorbance decrease between 0.008 and 0.1/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 (page 14).*
4. Initiate the reactions by adding 50 µl 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.*

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:

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

*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 340 nm can be determined using the NADPH extinction coefficient of 0.00373 μM^{-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 25°C.

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

*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 (0.6 cm).