ab83461

Glutathione Reductase (GR) Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Glutathione Reductase activity in various samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Glutathione Reductase (GR, EC 1.8.1.7) catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH), which plays an important role in the GSH redox cycle that maintains adequate levels of reduced GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress.

Abcam’s Glutathione Reductase Assay Kit is a highly sensitive, simple, direct and HTS-ready colorimetric assay for measuring GR activity in biological samples. In the assay, GR reduces GSSG to GSH, which reacts with 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) to generate TNB$^{2-}$ (yellow color, $\lambda_{\text{max}} = 405$ nm). The assay can detect 0.1-40 mU/ml GR in various samples.

2. Protocol Summary

Sample Preparation

↓

Sample Pre-Treatment

↓

Standard Curve Preparation

↓

Prepare and Add Reaction Mix

↓

Measure Optical Density
3. Components and Storage

A. Kit Components

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR Assay Buffer</td>
<td>100 mL</td>
</tr>
<tr>
<td>3 % H₂O₂</td>
<td>1 mL</td>
</tr>
<tr>
<td>Catalase (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>TNB Standard (2.5 µmol)</td>
<td>1 vial</td>
</tr>
<tr>
<td>DTNB (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>NADPH-GNERAT™ (Lyophilized)</td>
<td>2 vials</td>
</tr>
<tr>
<td>GSSG (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>GR Positive Control (10 mU; Lyophilized)</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

* Store kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Keep samples, NADPH-GNERAT™ solution and GR standard on ice during the assay. Read the entire protocol before performing the assay.
CATALASE: Dissolve with 1 mL Assay Buffer. The Catalase solution is stable for 1 week at 4°C and 1 month at -20°C.

TNB STANDARD: Dissolve with 0.5 mL Assay Buffer to generate 5 mM TNB Standard. The TNB standard solution is stable for 1 week at 4°C and 1 month at -20°C.

DTNB SOLUTION: Dissolve with 0.45 mL Assay Buffer, sufficient for 200 assays. The DTNB solution is stable for 2 weeks at 4°C and 1 month at -20°C.

NADPH-GERAT™: Dissolve with 0.22 mL Assay Buffer; sufficient for 100 assays. The solution is stable for 10 hours at 4°C and 2 weeks at -20°C.

GSSG: Dissolve with 1.3 mL Assay Buffer, sufficient for 200 assays. The GSSG solution is stable for 2 weeks at 4°C and 2 months at -20°C.

GR POSITIVE CONTROL: Dissolve lyophilized GR into 100 μL Assay Buffer, aliquot into vials, store at -20°C. It is stable for 1 day at 4°C and 1 month at -20°C.
B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker
4. Assay Protocol

1. Sample Preparation:
Homogenize 0.1 gram tissues on ice in 0.5-1.0 mL cold assay buffer, or 1 x 10^6 cells, or 0.2 mL Erythrocytes on ice in 0.1-0.2 mL cold assay buffer. Centrifuge at 10,000 x g for 15 min at 4°C. Collect the supernatant for assay and store on ice, serum can be tested directly. Store at -80°C.

2. Sample Pre-Treatment:
Samples should be treated to destroy GSH before the assay. Take 100 μL sample, add 5 μL 3% H_2O_2, mix and incubate at 25°C for 5 min. Then add 5 μL of Catalase, mix and incubate at 25°C for another 5 min. Add 2-50 μL of the pre-treated samples into a 96-well plate, bring the volume to 50 μL with Assay Buffer.

We suggest testing several doses of your sample to make sure the readings are within the standard curve range. As a guideline:

- **Cell lysates**: use a volume that corresponds to 1-5x10^5 cells per well,
- **Tissue lysates**: 10-50 μg of extracted protein per well
- **Biological fluids**: undiluted

Use 10 μL/well Positive Control (optional) and adjust to 50 μL with Assay Buffer.
3. **TNB Standard Curve:**
Add 0, 2, 4, 6, 8, 10 μL of the TNB Standard into 96-well plate in duplicate to generate 0, 10, 20, 30, 40, 50 nmol/well standard. Bring the final volume to 100 μL with Assay Buffer.
Optional. To control for background levels in samples with colour,, measure OD$_{405\text{nm}}$ at T$_0$ before adding reaction mix. OD$_{405\text{nm}}$ values above sample buffer should be subtracted from the result values from readings A$_1$ and A$_2$.

4. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 μl Reaction Mix:

- GR Assay Buffer: 40 μL
- DTNB solution: 2 μL
- NADPH-GNERAT™ solution: 2 μL
- GSSG solution: 6 μL

Add 50 μL of the Reaction Mix to each test samples. Mix well.

**Note. Wells with standard do not need Reaction Mix.**

5. **Immediately measure OD$_{405\text{nm}}$ at T$_1$ (reading A$_1$). Incubate the reaction at 25°C for 10 min (or incubate longer time if the GR activity is low), protect from light, measure OD$_{405\text{nm}}$ again at T$_2$ (reading A$_2$).**

$$\Delta A_{405\text{nm}} = A_2 - A_1.$$  

**Note:**
It is essential to read A$_1$ and A$_2$ in the reaction linear range. It will be more accurate if you read the reaction kinetics, and ensure A$_1$ and A$_2$ are in the reaction linear range.
5. Data Analysis

Plot the TNB standard Curve. Apply the \( \Delta A_{405nm} \) to the TNB standard curve to get \( \Delta B \) nmol of TNB.

\[
\text{GR Activity} = \frac{\Delta B}{(T_2 - T_1) \times 0.9 \times V} \times \frac{\text{Sample Dilution}}{\text{Factor}} = \text{nmol/min/ml} = \text{mU/ml}
\]

Where:

- \( \Delta B \) is the TNB amount from TNB standard Curve (in nmol).
- \( T_1 \) is the time of the first reading \( (A_1) \) (in min).
- \( T_2 \) is the time of the second reading \( (A_2) \) (in min).
- \( V \) is the pretreated sample volume added into the reaction well (in mL).
- 0.9 is the sample volume change factor during sample pre-treatment procedure.

**Unit Definition:** One unit is defined as the amount of enzyme that generates 1.0 \( \mu \text{mol} \) of TNB per minute at 25°C. The oxidation of 1 mole of NADPH to NADP\(^+\) will generate 2 mol TNB finally, therefore, 1 TNB unit equals 0.5 NADP unit.
TNB Standard Curve

\[ y = 0.036x + 0.0626 \]
\[ R^2 = 0.9992 \]

GR assay time line

O.D. 405 nm

O.D. 450nm

Time (minutes)
## 6. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Assay buffer at wrong temperature</td>
<td>Assay buffer must not be chilled - needs to be at RT</td>
</tr>
<tr>
<td>Protocol step missed</td>
<td></td>
<td>Re-read and follow the protocol exactly</td>
</tr>
<tr>
<td>Plate read at incorrect wavelength</td>
<td></td>
<td>Ensure you are using appropriate reader and filter settings (refer to datasheet)</td>
</tr>
<tr>
<td>Unsuitable microtiter plate for assay</td>
<td></td>
<td>Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells</td>
</tr>
<tr>
<td>Unexpected results</td>
<td>Measured at wrong wavelength</td>
<td>Use appropriate reader and filter settings described in datasheet</td>
</tr>
<tr>
<td>Samples contain impeding substances</td>
<td></td>
<td>Troubleshoot and also consider deproteinizing samples</td>
</tr>
<tr>
<td>Unsuitable sample type</td>
<td></td>
<td>Use recommended samples types as listed on the datasheet</td>
</tr>
<tr>
<td>Sample readings are outside linear range</td>
<td></td>
<td>Concentrate/ dilute samples to be in linear range</td>
</tr>
</tbody>
</table>
### Samples with inconsistent readings

<table>
<thead>
<tr>
<th>Samples with inconsistent readings</th>
<th>Unsuitable sample type</th>
<th>Refer to datasheet for details about incompatible samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples prepared in the wrong buffer</td>
<td>Use the assay buffer provided (or refer to datasheet for instructions)</td>
<td></td>
</tr>
<tr>
<td>Samples not deproteinized (if indicated on datasheet)</td>
<td>Use the <strong>10kDa spin column (ab93349)</strong></td>
<td></td>
</tr>
<tr>
<td>Cell/ tissue samples not sufficiently homogenized</td>
<td>Increase sonication time/ number of strokes with the Dounce homogenizer</td>
<td></td>
</tr>
<tr>
<td>Too many freeze-thaw cycles</td>
<td>Aliquot samples to reduce the number of freeze-thaw cycles</td>
<td></td>
</tr>
<tr>
<td>Samples contain impeding substances</td>
<td>Troubleshoot and also consider deproteinizing samples</td>
<td></td>
</tr>
<tr>
<td>Samples are too old or incorrectly stored</td>
<td>Use freshly made samples and store at recommended temperature until use</td>
<td></td>
</tr>
</tbody>
</table>

### Lower/ Higher readings in samples and standards

<table>
<thead>
<tr>
<th>Lower/ Higher readings in samples and standards</th>
<th>Not fully thawed kit components</th>
<th>Wait for components to thaw completely and gently mix prior use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Out-of-date kit or incorrectly stored reagents</td>
<td>Always check expiry date and store kit components as recommended on the datasheet</td>
<td></td>
</tr>
<tr>
<td>Reagents sitting for extended periods on ice</td>
<td>Try to prepare a fresh reaction mix prior to each use</td>
<td></td>
</tr>
<tr>
<td>Incorrect incubation time/ temperature</td>
<td>Refer to datasheet for recommended incubation time and/ or temperature</td>
<td></td>
</tr>
<tr>
<td>Incorrect amounts used</td>
<td>Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)</td>
<td></td>
</tr>
<tr>
<td>Problem</td>
<td>Reason</td>
<td>Solution</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>--------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Standard curve is not linear</td>
<td>Not fully thawed kit components</td>
<td>Wait for components to thaw completely and gently mix prior use</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors when setting up the standard curve</td>
<td>Try not to pipette too small volumes</td>
</tr>
<tr>
<td></td>
<td>Incorrect pipetting when preparing the reaction mix</td>
<td>Always prepare a master mix</td>
</tr>
<tr>
<td></td>
<td>Air bubbles in wells</td>
<td>Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates</td>
</tr>
<tr>
<td></td>
<td>Concentration of standard stock incorrect</td>
<td>Recheck datasheet for recommended concentrations of standard stocks</td>
</tr>
<tr>
<td></td>
<td>Errors in standard curve calculations</td>
<td>Refer to datasheet and re-check the calculations</td>
</tr>
<tr>
<td></td>
<td>Use of other reagents than those provided with the kit</td>
<td>Use fresh components from the same kit</td>
</tr>
</tbody>
</table>
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