Glutathione Peroxidase (GSH-Px) Activity Assay Kit

Catalog No: E-BC-K096-M
Method: Colorimetric method
Specification: 96T (Can detect 40 samples without duplication)
Measuring instrument: Microplate reader
Sensitivity: 34.34 U
Detection range: 34.34-1036.64 U

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA)    Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.
Application
This kit can be used to measure GSH-Px activity of animal serum, plasma, tissue, cells, cell culture supernatant and other samples.

Detection significance
Glutathione Peroxidase (GSH-Px) is an important enzyme that catalyzes decomposition of hydrogen peroxide. GSH specifically catalyze the reaction between GSH and hydrogen peroxide, protecting cell membrane structure and keeping membrane function integrity. Se-cysteine is the active center of the GSH-Px. Determination of GSH-Px activity in organism can be an indicator of selenium level as Se is essential section of GSH-Px.

Detection principle
Glutathione Peroxidase (GSH-Px) can promote the reaction of hydrogen peroxide (H_2O_2) and reduced glutathione to produce H_2O and oxidized glutathione (GSSG). The activity of glutathione peroxidase can be expressed by the rate of enzymatic reaction. The activity of glutathione can be calculated by measuring the consumption of reduced glutathione. Hydrogen peroxide (H_2O_2) and reduced glutathione can react without catalysis of GSH-Px, so the portion of GSH reduction by non-enzymatic reaction should be subtracted. GSH can react with dinitrobenzoic acid to produce 5-thio-dinitrobenzoic acid anion, which showed a stable yellow color. Measure the absorbance at 412nm, and calculate the amount of GSH.

\[
\text{GSH-PX} \\
\text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow 2\text{H}_2\text{O} + \text{GSSG} \\
\text{GSH} + \text{DTNB} \rightarrow \text{GSSG} + \text{TNB}
\]

Experimental instrument
Test tube
Micropipettor
Vortex mixer
Centrifuge
37°C water bath/gas bath
Microplate reader (412 nm)
Kit components

<table>
<thead>
<tr>
<th>Item</th>
<th>Component</th>
<th>Specifications</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>Stock Solution</td>
<td>0.5 mL × 1 vial</td>
<td>2-8°C, 6 months</td>
</tr>
</tbody>
</table>

**Reagent 1 application solution:** dilute the Reagent 1 with double distilled water at ratio of 1:99. Prepare fresh solution before use. Store at 4°C.

<table>
<thead>
<tr>
<th>Reagent 2</th>
<th>Acid Reagent</th>
<th>50 mL × 1 vial</th>
<th>2-8°C, 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 3</td>
<td>Phosphate</td>
<td>12 mL × 1 vial</td>
<td>2-8°C, 6 months</td>
</tr>
<tr>
<td>Reagent 4</td>
<td>DTNB Solution</td>
<td>7 mL × 1 vial</td>
<td>2-8°C, 6 months, shading light</td>
</tr>
<tr>
<td>Reagent 5</td>
<td>GSH Standard</td>
<td>3.07 mg × 1 vial</td>
<td>2-8°C, 6 months</td>
</tr>
<tr>
<td>Reagent 6</td>
<td>GSH Standard Stock Diluent</td>
<td>1.5 mL × 2 vials</td>
<td>2-8°C, 6 months</td>
</tr>
</tbody>
</table>

**GSH standard application solution:** dilute the reagent 6 with double distilled water at a ratio of 1:10. Prepare fresh solution before use.

**1 mmol/L GSH standard solution:** dissolve a vial of GSH standard with GSH standard application solution to a final volume of 10 mL before use and mix fully. Prepare fresh solution before use.

**100 μmol/L GSH standard solution:** dilute 1 mmol/L GSH standard solution with GSH standard application solution at ratio of 1:9 and mix fully.

**Note:** Bring all reagents to room temperature before use.

Sample preparation

1. **Serum (Plasma):**
   Detect the sample directly. If the concentration is beyond the linear range, then dilute the sample with normal saline before detection.

2. **10% tissue homogenate:**
   Accurately weigh the tissue weight, add 9 times the volume of homogenized medium according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min at 4°C, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

3. **Cell sample:**
   Collect the 1×10^6 cells and centrifuge at 1000~1500 g for 10 min. Discard the supernatant and keep the cell sediment. Add PBS at a ratio of cell number (10^6): PBS (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min at 4°C, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).
Pre-experiment

1. Determine optimal dilution factor of samples before formal experiment. Samples are diluted into different concentration with ddH₂O, then take the pre-experiment according to the operation procedures. Calculate the inhibition ratio of serial diluent, and choose the optimal dilution factor when inhibition ratio in the range of 25%-45%.

\[
\text{Inhibition ratio} = \frac{\text{OD}_{\text{Non-enzyme tube}} - \text{OD}_{\text{Enzyme tube}}}{\text{OD}_{\text{Non-enzyme tube}}} \times 100\%
\]

2. The optimal dilution factor of sample are different for different species, the GSH-Px also are different for different samples. So it is best to do a preliminary experiment to determine the optimal dilution factor for a new sample.

3. It is best to reserve 3 paralleled tubes with different dilution factor of sample in preliminary experiment for determining the optimal dilution factor. For example, Tissue homogenate: Pipet 20 μL of 5%, 1%, 0.5%, 0.4%, 0.3%, 0.1% tissue homogenate supernatant respectively for the assay. Serum: Dilute serum samples with normal saline at ratio of 1:1, 1:4, 1:7, 1:9 respectively. Then pipet 20 μL of undiluted and diluted serum samples for the assay.

4. Adjust dilution factor: If inhibition ratio > 50%, need to further dilute the sample then process the test. If inhibition ratio < 10%, need to increase sample concentration.

Operation steps

1. The preparation of standard curve

Dilute 100 μmol/L GSH standard solution with GSH standard application solution to a serial concentration. The recommended dilution gradient is as follows: 100, 80, 60, 50, 40, 20, 10, 0 μmol/L.

2. Enzymatic reaction (Reagent 1 application solution is preheated in advance at 37℃)

<table>
<thead>
<tr>
<th></th>
<th>Non-enzyme tube</th>
<th>Enzyme tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mmol/L GSH (μL)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Sample (μL)</td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

Pre-heat the tubes at 37℃ water bath for 5 min. Preheat Reagent 1 application solution at 37℃ for 5 min at the same time.

<table>
<thead>
<tr>
<th>Reagent 1 application solution (μL)</th>
<th>10</th>
<th>10</th>
</tr>
</thead>
</table>

React at 37℃ water bath for 5 min accurately.

<table>
<thead>
<tr>
<th>Reagent 2 (μL)</th>
<th>200</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample (μL)</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

Mix fully and centrifuge at 3100 g for 10 min, then take 100 μL of supernatant for chromogenic reaction.
3. Chromogenic reaction

<table>
<thead>
<tr>
<th></th>
<th>Standard well</th>
<th>Non-enzyme well</th>
<th>Enzyme well</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH standard solution with different concentrations (μL)</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant (μL)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Reagent 3 (μL)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Reagent 4 (μL)</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Mix fully and stand for 5 min at room temperature. Measure the OD values of each well at 412 nm with microplate reader.

Calculation of results

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: $y = ax + b$.

1. For serum/plasma sample and cell culture supernatant sample:
   **Definition:** The amount of GSH-Px in 0.1 mL of sample that catalyze the consumption of 1 μmol/L GSH with deducting the effect of non-enzyme reaction at 37°C for 5 minute is defined as 1 unit.
   
   \[
   \text{GSH-Px activity (U)} = \left( \Delta A_{412} - b \right) \div a \times \frac{0.23 + V}{0.03 + V} \times 0.1^* \times f
   \]

2. For tissue and cells sample:
   **Definition:** The amount of GSH-Px in 1 mg of protein that catalyze the consumption of 1 μmol/L GSH with deducting the effect of non-enzyme reaction at 37°C for 5 minute is defined as 1 unit.
   
   \[
   \text{GSH-Px activity (U/mgprot)} = \left( \Delta A_{412} - b \right) \div a \times \frac{0.23 + V}{0.03 + V} \times \left( V \times C_{pr} \right) \times f
   \]

[Note]

y: The absolute OD value of standard;
x: The concentration of standard;
a: The slope of standard curve;
b: The intercept of standard curve.
$\Delta A_{412}$: The absolute OD value of sample (OD_{Non-enzyme tube} - OD_{Enzyme tube}).
$(0.23 + V)/(0.03 + V)$: Dilution factor of sample in enzymatic reaction.
0.1*: The volume of sample in definition.
V: The volume of sample added to the reaction system.
f: Dilution factor of sample before tested.
$C_{pr}$: Concentration of protein in sample (mgprot/mL)
Technical parameter
1. The sensitivity of the kit is 34.34 U.
2. The intra-assay CV is 2.4% and the inter-assay CV is 8.7%.
3. The recovery of the kit is 104%.
4. The detection range of the kit is 34.34-1036.64 U.

Notes
1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.
5. The supernatant after centrifugation after adding reagent 2 in enzymatic reaction must be clarified.
6. Determine optimal dilution factor of samples before formal experiment. It is recommended to choose the optimal dilution factor when inhibition ratio in the range of 25%-45%.
7. Reagent 1 application solution should be preheated in advance at 37°C

Appendix: Standard curve
(This is for reference only.)