Glutathione-S-Transferase Fluorometric/Colorimetric Assay Kit

Catalog No. KM0081

Detection and Quantification of Glutathione-S-Transferase Concentrations in Biological Samples.

Research Purposes Only. Not Intended for Diagnostic or Clinical Procedures.
INTRODUCTION

Glutathione S-transferase or GSTs are composed of a variety of cytosolic, mitochondrial and microsomal proteins. These enzymes are present in eukaryotes and prokaryotes where they facilitate a series of reactions and are able to accept endogenous and xenobiotic substrates. GSTs are able to catalyze the conjugation of reduced glutathione through a sulfhydryl group to electrophilic centers on many types of substrates. Through this, endogenous compounds are able to detoxify and xenobiotic substrates are able to break down. Moreover, they are able to bind toxins and function as transporters. Structurally, mammalian cytosolic GSTs are dimeric where both subunits are known to be of the same class of GSTs. Each monomer consists of about 22-30 kDa and are active over many types of substrates with overlap. In terms of disease, genetic polymorphisms in glutathione S-transferase and its altered expression and activity are associated with oxidative DNA damage and also damage of the kidney, causing the individual’s risk of cancer susceptibility to increase.

In this assay, Glutathione-S-Transferase, in the presence of reduced glutathione (GSH), reacts with a dye reagent to produce a colored product that can be detected by absorbance at 340 nm.

FEATURES

Number of Assays: 100 Assays
 Samples: serum, plasma, urine, milk, saliva, cell culture, etc.
 Note: This assay kit is validated with serum.
 Colorimetric Dynamic Range: 50 mU/ml to 500 mU/ml Glutathione-S-Transferase Absorbance at 340 nm

STORAGE & HANDLING

Upon receipt, take out Reaction Buffer and store at 4°C. Take out the microplate and leave at room temperature. Store all other components in the original box at -20°C, protected from light. The kit should be stable for at least six months.

Note: Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.
KIT CONTENTS & STOCK PREPARATION

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
<th>Storage</th>
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<tbody>
<tr>
<td>96-Well Microplate</td>
<td>1 plate</td>
<td>RT</td>
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<tr>
<td>Reaction Buffer</td>
<td>25 ml</td>
<td>4°C</td>
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<tr>
<td>Glutathione-S-Transferase Standard (Blue Cap)</td>
<td>1 vial, lyophilized</td>
<td>-20°C</td>
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<td>Reaction Mix (Green Cap)</td>
<td>1 vial, lyophilized</td>
<td>-20°C</td>
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<tr>
<td>Dye Reagent (Red Cap)</td>
<td>70 µl</td>
<td>-20°C</td>
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**Note:** If not running entire plate at once, aliquot and store reagents under conditions according to this table. Use within 2 months.

MATERIALS NOT PROVIDED

Pipetting devices, tubes, and microplate reader.

ASSAY RESTRICTIONS

- This kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.
- Materials included in this kit should NOT be used past the expiration date on the kit label.
- Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.
- Variations in pipetting technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in performance of the materials provided.
- The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.
REAGENT & SAMPLE PREPARATIONS
Please read the manual in its entirety before proceeding with the assay.

Note: Warm all reagents to room temperature before use and protect from light. Calculate and prepare the amount needed if a full plate is not used.

1. Preparation of Standard
Reconstitute the vial of Glutathione-S-Transferase Standard in 500 ul of Reaction Buffer to make a stock concentration of 0.5 U/ml.

Colorimetric Method: To prepare the standard, dilute the appropriate amount of reconstituted Glutathione-S-Transferase stock solution in Reaction Buffer, starting from a high point of 500 mU/ml down to a low point of 50 mU/ml to create 7 concentrations for the standard. The last row consists of only Reaction Buffer to serve as a negative control. The standards are run in duplicate. Use a 96-well clear, flat bottom plate.

2. Preparation of Samples
Dilute the samples in Reaction Buffer. A variable dilution will be required depending on the total amount of Glutathione-S-Transferase present in your sample. In the first trial, the samples should be serially diluted to determine the optimal amount of sample for the assay. For unknown samples, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

3. Preparation of Reaction Mix
Reconstitute Reaction Mix with 5.95 ml of Reaction Buffer. If a full plate is used, put the reconstituted Reaction Mix on ice while in use. If not all of it is used, aliquot and store at -20°C. Avoid multiple freeze and thaw cycles.

4. Preparation of Working Solution
Add 50 µl of Dye Reagent in the 5.95 ml reconstituted Reaction Mix to make the Working Solution. Mix well by inversion. Put on ice and protect from light since this solution is temperature and light sensitive. This solution cannot be stored, so make the exact amount needed for the experiments.
ASSAY PROTOCOL

1. Add 50 µl of each sample/standard to each well in a 96-well clear, flat bottom plate. The standards are run in duplicate. It is advised to run the samples in duplicate or triplicate.

Sample Plate Layout (you do not have to follow this)

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Note: BL=Blank Control, ST=Glutathione-S-Transferase Standards, EX=Experimental Test Samples

2. Add 50 µl of the Working Solution to each well of the microplate containing the standards, controls, and samples.

3. Incubate at room temperature for 3 minutes, protected from light.

4. Read the plate using a microplate reader at 340 nm.
   Because the assay is continuous (not terminated), the plate can be measured at multiple time points to follow the kinetics of the reaction.
STANDARD CURVE

Correct for background fluorescence or absorbance. For each point, subtract the value derived from the negative control. Plot the Glutathione-S-Transferase concentrations vs. the read values to create the standard curve.

![Standard Curve Graph]

Glutathione-S-Transferase Fluorometric/Colorimetric Assay Kit allows for the detection and quantification of endogenous Glutathione-S-Transferase concentrations within the range of 50 mU/ml - 500 mU/ml in cell lysates, sera, and plasma.

CALCULATION

Determine the slope of the standard curve and calculate the Glutathione-S-Transferase concentration of your samples.

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[\text{Glutathione-S-Transferase}] = \left(\frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}}{\text{Slope}}\right) \times \text{Dilution Factor}
\]
TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.immunoway.com or contact us at tech@immunoway.com

NOTES