Technical Bulletin

Glutathione S-Transferase Assay Kit

Catalog Number MAK453

Product Description

Glutathione transferase (GST) is a multifunctional enzyme that plays an important role in cellular detoxification. GST protects cells against foreign compounds such as carcinogens and drugs by catalyzing the attachment of glutathione to the compounds electrophilic and/or hydrophobic sites.

The Glutathione S-transferase Assay Kit is based on the GST enzyme reaction between GSH and the GST substrate, CDNB (1-chloro-2,4-dinitrobenzene). The GST catalyzed formation of GS-DNB produces a dinitrophenyl thioether which can be detected spectrophotometrically at 340 nm. The rate of increase in absorbance at 340 nm is directly proportional to the GST activity in the sample. The linear detection range for the assay method is 2 to 80 U/L for a 10 minute reaction at 25 °C.

The kit is suitable for Glutathione S-transferase activity determination in cell lysates, tissues, etc.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 25 mL
  Catalog Number MAK453A
- Glutathione 1 vial
  Catalog Number MAK453B
- CDNB 120 µL
  Catalog Number MAK453C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates suitable for use with UV spectrophotometric readings. Cell culture or tissue culture treated plates are **not** recommended.
- Refrigerated microcentrifuge capable of RCF ≥ 10,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- 1.5 mL microcentrifuge tubes
- EDTA (Catalog Number ED2SS or equivalent)
- Phosphate Buffered Saline (Catalog Number P3813 or equivalent)
- Potassium phosphate monobasic (Catalog Number P0662 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Assays can be executed at any desired temperature (e.g., 25 °C or 37 °C). Bring all reagents to the desired reaction temperature (e.g., 25 °C) prior to use.

Glutathione: Reconstitute vial with 120 µL of purified water. Vortex to mix. Unused Glutathione Reagent is stable for three weeks when stored frozen at -20 °C.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Tissue

1. Prior to dissection, rinse tissue in phosphate buffered saline, pH 7.4, to remove blood.
2. Homogenize tissue (50 mg) with a Dounce homogenizer in 250 µL cold 100 mM potassium phosphate buffer, pH 7.0, containing 2 mM EDTA.
3. Freeze the homogenized tissue at -80 °C to lyse the cells.
4. After freezing, thaw and centrifuge samples at 10,000 × *g* for 15 minutes at 4 °C.
5. Remove supernatant and retain for assay.

Cell Lysate

1. Collect cells (~4 million) by centrifugation at 2,000 × *g* for 5 minutes at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman or cell scraper.
3. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 100 mM potassium phosphate buffer, pH 7.0, and 2 mM EDTA.
4. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
5. Remove supernatant and retain for assay.

All Samples

1. For unknown samples, test several dilutions.
2. All samples can be stored at -20 to -80 °C for at least one month.
3. Transfer 20 µL of each Sample to separate wells of a clear 96-well plate.

Working Reagent

Note: This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to Samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

1. Mix enough Working Reagent for the number of assays to be performed. For each well, prepare 186 µL of Working Reagent according to Table 1.

Table 1.
Preparation of Working Reagent

Reagent	Working Reagent
Assay Buffer	184 µL
Glutathione Reagent	1 µL
CDNB	1 µL

2. Add 180 µL of Working Reagent to each well. Tap plate briefly to mix.

Measurement

Immediately measure optical density at 340 nm (OD_{340}) in kinetic mode every minute for 10 minutes at desired reaction temperature (e.g., 25 °C or 37 °C).

Results

1. Plot the OD_{340} versus time for each well.

2. Choose two time points (T_1 and T_2) in the linear portion of the curve (OD_{T2} and OD_{T1}) to determine the GST activity of Sample as follows:

GST (U/L) =

$$\frac{OD_{T2}-OD_{T1}}{T} \text{ x } \frac{1}{0.0096\ \mu M^{-1}cm^{-1}\ x\ l} \text{ x } \frac{V_{Total}}{V_{Sample}} \times DF$$

=

$$\frac{OD_{T2}-OD_{T1}}{T} \text{ x } \frac{10}{0.00503\ \mu M^{-1}} \times DF$$

where

OD_{T1} = Optical density (OD_{340}) of the first time point (T_1) chosen in the linear portion of the curve

OD_{T2} = Optical density (OD_{340}) of the second time point (T_2) chosen in the linear portion of the curve

0.0096 $\mu M^{-1}cm^{-1}$ = Extinction coefficient of GS-DNB

l = Path-length for 200 µL in a 96-well plate (0.524 cm)

0.00503 μM^{-1} = Extinction coefficient of GS-DNB × l (0.0096 $\mu M^{1-}cm^{-1}$ × 0.524 cm)

T = Time difference (in minutes) between OD_{T2} and OD_{T1}

V_{Total} = Total reaction volume (200 µL)

V_{Sample} = Sample volume (20 µL)

DF = Sample Dilution factor (DF = 1 for undiluted Samples)

Unit definition: One unit of Glutathione S-transferase will conjugate 1 µmole of CDNB per minute under the assay conditions.

Figure 1.
Kinetics of GST Reaction in a 96-Well Plate

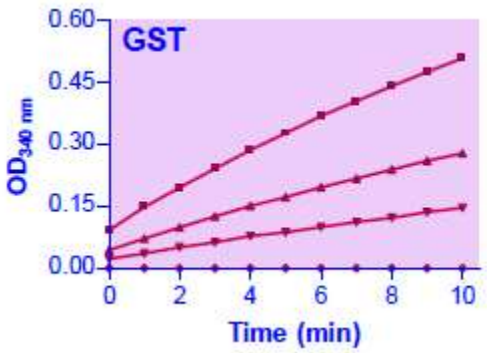
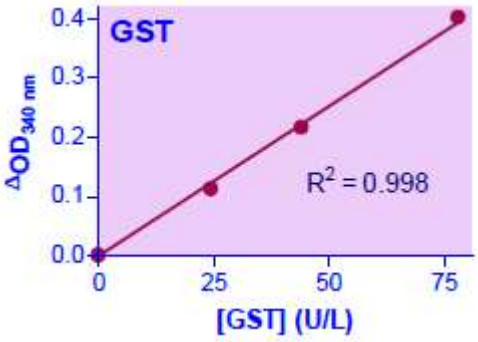


Figure 2.
Typical GST Titration Curve.



References

1. Csiszár, J., et al., Glutathione transferase supergene family in tomato: Salt stress-regulated expression of representative genes from distinct GST classes in plants primed with salicylic acid. *Plant Physiol. Biochem.,* **78**, 15-26 (2014).
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3. Watson, M.A., et al., Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis,* **19**, 275-280 (1998).

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