Glutathione S-Transferase Assay Kit

Catalog Number KA1316

96 assays

Version: 03

Intended for research use only

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Introduction

Background

Glutathione S-transferases (GSTs) are ubiquitous multifunctional enzymes, which play a key role in cellular detoxification. The enzymes protect cells against toxicants by conjugating them to glutathione, thereby neutralizing their electrophilic sites, and rendering the products more water-soluble.[1] The glutathione conjugates are metabolized further to mercapturic acid and then excreted.[1] Based on their sequence homology, substrate specificity and immunological cross-reactivity, GSTs have been grouped into species-independent classes of isozymes.[2-5] These classes are comprised of both cytosolic and microsomal enzymes.[2-5]

Principle of the Assay

Glutathione S-Transferase Assay Kit measures total GST activity (cytosolic and microsomal) by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione.[6] The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample. The GST Assay Kit can be used to measure GST activity in plasma, erythrocyte lysates, tissue homogenates, and cell lysates. Cytosolic and microsomal GST activity can be assayed separately following the procedure outlined under Sample Preparation.

General Information

Materials Supplied

List of component

Item	Quantity
GST Assay Buffer (2X)	1 vial
GST Sample Buffer (2X)	1 vial
GST Assay (control)	1 vial
GST Glutathione	1 vial
GST CDNB	1 vial
96-Well Plate (Colorimetric Assay)	1 plate
96-Well Cover Sheet	1 cover

Storage Instruction

This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Materials Required but Not Supplied

- ✓ A plate reader capable of measuring absorbance at 340 nm
- ✓ Adjustable pipettors and a repeating pipettor
- ✓ A source of pure water; glass distilled water or HPLC-grade water is acceptable

Precautions for Use

Please read these instructions carefully before beginning this assay.
WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

- Pipetting Hints
- ✓ It is recommended that an adjustable pipette be used to deliver Assay Buffer (dilute), glutathione, and CDNB to the wells.
- ✓ Use different tips to pipette the Assay Buffer (dilute), glutathione, enzyme, and CDNB.
- ✓ Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- ✓ Do not expose the pipette tip to the reagent(s) already in the well.

Assay Protocol

Reagent Preparation

- ✓ GST Assay Buffer (2X) - Dilute 10 mL of GST Assay Buffer concentrate with 10 mL of HPLC-grade water. This final Assay Buffer (100 mM potassium phosphate, pH 6.5, containing 0.1% Triton X-100) should be used in the assay. When stored at 4°C, this diluted Assay Buffer is stable for at least two months. Equilibrate the diluted Assay Buffer to 25°C before using in the assay.
- ✓ GST Sample Buffer (2X) - Dilute 5 mL of GST Sample Buffer concentrate with 5 mL of HPLC-grade water. This final Sample Buffer (100 mM potassium phosphate, pH 6.5, containing 0.1% Triton X-100, and 1 mM glutathione) should be used to dilute the GST control and the GST samples prior to assaying. When stored at 4°C, this diluted Sample Buffer is stable for at least one month.
- ✓ GST Assay (control) - This vial contains a solution of equine liver GST. To avoid repeated freezing and thawing, the GST should be aliquoted into several small vials and stored at -20°C. Prior to use, transfer 10 µL of the supplied enzyme to another vial and dilute with 190 µL of diluted Sample Buffer and keep on ice. The diluted enzyme is stable for four hours on ice. A 20 µL aliquot of this diluted enzyme per well causes an increase of approximately 0.04 absorbance unit/minute under the standard assay conditions described in Performing the Assay.
- ✓ GST Glutathione - This vial contains a solution of reduced glutathione and should be stored at -20°C when not being used. The reagent is ready to use as supplied.
- ✓ GST CDNB - This vial contains an ethanolic solution of 1-chloro-2,4-dinitrobenzene (CDNB) and should be stored at -20°C when not being used. The reagent is ready to use as supplied. Equilibrate the reagent to 25°C before using in the assay.

- Plate Set Up
- ✓ There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as non-enzymatic or background wells. The absorbance rate of these wells must be subtracted from the absorbance rate measured in the GST sample and control wells. We suggest that there be at least three wells designated as positive controls and that you record the contents of each well on Plate Layout.

Sample Preparation

The procedures listed below for tissue homogenates and cell lysates will result in assaying total GST activity (cytosolic and microsomal). To separate the two enzymes, centrifuge the 10,000 x g supernatant at 100,000 x g for 60 minutes at 4°C. The resulting 100,000 x g supernatant will contain cytosolic GST and the pellet will contain microsomal GST. Suspend the microsomal pellet in cold buffer (*i.e.*, 100 mM potassium phosphate, pH 7.0, containing 2 mM EDTA). If not assaying on the same day, freeze the samples at -80°C.

- Tissue Homogenate

1. Prior to dissection, perfuse or rinse tissue with a PBS (phosphate buffered saline) solution, pH 7.4, to remove any red blood cells and clots.
2. Homogenize the tissue in 5-10 mL of cold buffer (*i.e.*, 100 mM potassium phosphate, pH 7.0, containing 2 mM EDTA) per gram tissue
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.

- Cell Lysate

1. Collect cells by centrifugation (*i.e.*, 1,000-2,000 x g for 10 minutes at 4°C). For adherent cells, do not harvest using proteolytic enzymes; rather use a rubber policeman.
2. Homogenize or sonicate cell pellet in 1-2 mL of cold buffer (*i.e.*, 100 mM potassium phosphate, pH 7.0, containing 2 mM EDTA).
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.

- Plasma and Erythrocyte Lysate

1. Collect blood using an anticoagulant such as heparin, citrate, or EDTA.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice until assaying or freeze at -80°C. The plasma sample will be stable for at least one month.
3. Remove the white buffy layer (leukocytes) and discard.
4. Lyse the erythrocytes (red blood cells) in four times its volume of ice-cold HPLC-grade water.
5. Centrifuge at 10,000 x g for 15 minutes at 4°C.
6. Collect the supernatant (erythrocyte lysate) for assaying and store on ice. If not assaying the same day, freeze at -80°C. The sample will be stable for at least one month.

- Tissue Homogenization using the Precellys 24 Homogenizer

1. Prior to dissection, either perfuse or rinse tissue with phosphate buffered saline (PBS), pH 7.4, to remove any red blood cells and clots.
2. Freeze organs immediately upon collection and then store at -80°C. Snap-freezing of tissues in liquid nitrogen is preferred.
3. Add cold 100 mM potassium phosphate, pH 7.0, containing 2 mM EDTA.
4. Homogenize tissue sample using the Precellys 24 according to appropriate settings.
5. Spin the tissue homogenates at 10,000 x g for 15 minutes at 4°C.
6. Collect supernatant and assay samples according to the kit booklet protocol. Samples may need to be diluted appropriately for assay and should be normalized using a protein assay.

Assay Procedure

- ✓ The final volume of the assay is 200 µL in all the wells.
- ✓ It is not necessary to use all the wells on the plate at one time.
- ✓ The assay temperature is 25°C.
- ✓ Use the Assay Buffer (dilute) in the assay.
- ✓ Monitor the increase in absorbance at 340 nm using a plate reader.

1. Background or Non-enzymatic Wells - add 170 µL of Assay Buffer and 20 µL of Glutathione to three wells.
2. Positive Control Wells (equine liver GST) - add 150 µL of Assay Buffer, 20 µL of Glutathione, and 20 µL of diluted GST (control) to three wells.
3. Sample Wells - add 150 µL of Assay Buffer, 20 µL of Glutathione, and 20 µL of sample to three wells. To obtain reproducible results, the amount of GST added to the well should cause an absorbance increase between 0.012 and 0.064/min. When necessary, samples should be diluted with Sample Buffer or concentrated with an Amicon centrifuge concentrator with a molecular weight cut-off of 3,000 to bring the enzymatic activity to this level. *NOTE: The amount of sample added to the well should always be 20 µL.*
4. Initiate the reactions by adding 10 µL of CDNB to all the wells you are using. Make sure to note the precise time you started and add the CDNB 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.

Data Analysis

Calculation of Results

- Determination of the Reaction Rate

✓ 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 in Figure 1 using equine liver GST) *-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:

$$\Delta A_{340}/\text{min.} = \frac{A_{340}\,(\text{Time 2}) - A_{340}\,(\text{Time 1})}{\text{Time 2}\,(\text{min.}) - \text{Time1}\,(\text{min.})}$$

✓ Determine the rate of ΔA_{340}/min. for the background or non-enzymatic wells and subtract this rate from that of the sample wells.

✓ Use the following formula to calculate the GST activity. The reaction rate at 340 nm can be determined using the CDNB extinction coefficient of 0.00503 μM^{-1}*. One unit of enzyme will conjugate 1.0 nmol of CDNB with reduced glutathione per minute at 25°C.

$$\text{GST Activity} = \frac{\Delta A_{340}/\text{min.}}{0.00503\,\mu\text{M}^{-1}} \times \frac{0.2\,\text{mL}}{0.02\,\text{mL}} \times \text{Sample dilution} = \text{nmol/min/mL}$$

*The actual extinction coefficient for CDNB at 340 nm is 0.0096 $\mu M^{-1}cm^{-1}$.[6] This value has been adjusted for the pathlength of the solution in the well (0.524 cm).

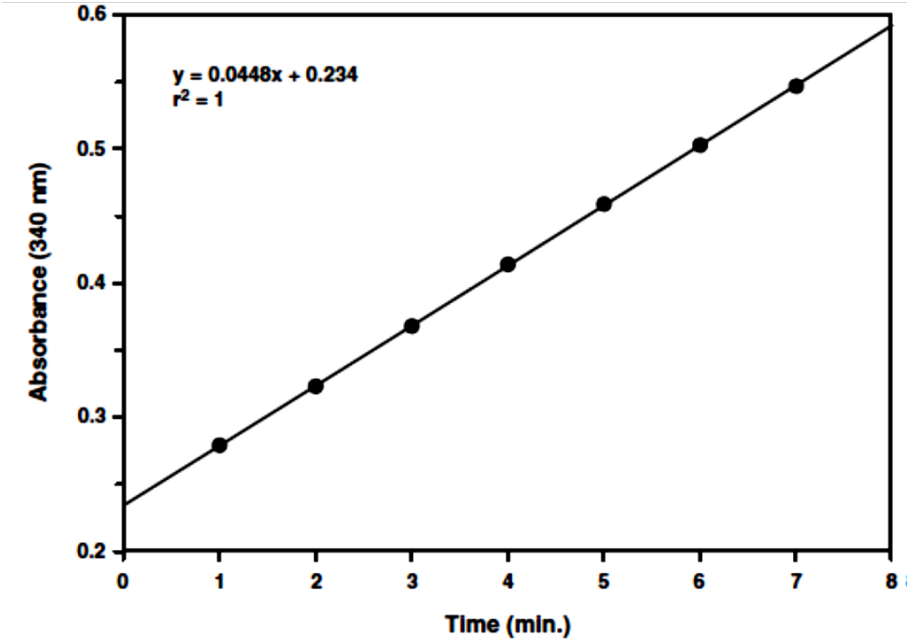

Figure 1. Activity of equine liver GST

Performance Characteristics

- Precision

When a series of eight GST samples were assayed on the same day, the intra-assay coefficient of variation was 3.6%. When a series of eight GST samples were assayed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 4.8%.

- Sensitivity

The dynamic range of the assay is limited only by the accuracy of the absorbance measurement. Most plate readers are linear to an absorbance of 1.2. Samples containing GST activity between 24-128 nmol/min/mL can be assayed without further dilution or concentration. This GST activity is equivalent to an absorbance increase of 0.012 to 0.064 per minute.

- Interferences and Inhibitors

✓ Samples that have a high intrinsic absorbance at 340 nm may exceed the absorbance maximum of the plate reader. Therefore, samples with an initial absorbance >0.7 should be diluted with Sample Buffer until the absorbance is lowered. For example, hemoglobin absorbs significantly at 340 nm, and thus erythrocyte lysates must be diluted before assaying.

✓ Inhibitors can be dissolved in dimethylsulfoxide or ethanol but no more than 10 µL should be added to the assay. The volume of Assay Buffer in the well should be adjusted so as to accommodate the inhibitor volume. The final volume in the wells should be 200 µL. The inhibitor should be added to the wells before the reaction is initiated with CDNB. The 100% Initial Activity well should contain GST and 10 µL of solvent (inhibitor solvent).

✓ The following reagents were tested for interference in the assay.

	Reagent	Will Interfere (Yes or No)
Buffers:	Tris	No
	Phosphate	No
Detergents:	Triton X-100 (≤0.1%)	No
	Tween 20 (0.1%)	Yes
	CHAPS (0.1%)	Yes
Protease Inhibitors/ Chelators:	Antipain (0.1 mg/mL)	Yes
	Leupeptin (10 µg/mL)	Yes
	Trypsin (10 µg/mL)	Yes
	Chymostatin (10 µg/mL)	Yes
	PMSF (≤200 µM)	No
	EGTA (≤5 mM)	No
	EDTA (≤2 mM)	No
Solvents:	Ethanol (10 µL)	No
	Methanol (10 µL)	Yes
	Dimethyl Sulfoxide (10 µL)	No
Others:	Glycerol (≤10%)	No
	BSA (≤1%)	No

Resources

Trouble shooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
The initial absorbance in the wells is less than 0.1	CDNB was not added to the wells	Make sure to add all components to the wells and re-assay
No increase in absorbance was observed in the sample wells	Enzyme activity was too low or glutathione was not added to the wells	Concentrate your sample by using an Amicon centrifuge concentrator with a 3,000 MW cut-off and re-assay. Make sure to add components to the wells.
Reaction rate was too fast	Too much enzyme added to well(s)	Dilute your samples with Sample Buffer and re-assay
The initial absorbance in the sample wells is above 0.7		Dilute your samples with Sample Buffer and re-assay

References

1. Boyland, E. and Chasseaud, L.F. The role of glutathione and glutathione S-transferases in mercapturic acid biosynthesis. *Adv. Enzymol.* 32, 173-219 (1969).
2. Mannervik, B. The isoenzymes of glutathione transferase. *Adv. Enzymol. Relat. Areas Mol. Biol.* **57**, 357-417 (1985).
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4. Morgenstern, R., DePierre, J.W., and Ernster, L. Activation of microsomal glutathione S-transferase activity by sulfhydryl reagents. *Biochem. Biophys. Res. Commun.* **87**, 657-663 (1979).
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6. Habig, W.H., Pabst, M.J., and Jakoby, W.B. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **249**, 7130-7139 (1974)

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H