Glutathione S-Transferase (GST) Fluorometric Assay kit (96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-33103-96

www.zellx.de

Sample Types Validated for:

Serum, Plasma, Urine and Cell Lysates

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

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Please read this insert completely prior to using the product.

ZELLX®
Krantorweg 48c
13503 Berlin
Tel.: +49(0)30 81309085
Fax: +49(0)30 81309086
E-Mail: support@zellx.de
Web: www.zellx.de

Introduction

Background

The Glutathione S-Transferase (GST) family of isozymes are expressed in almost all tissues, and function to detoxify a wide variety of electrophilic molecules by mediating their conjugation with reduced glutathione.

Due to its pivotal role in ameliorating oxidative stress, the GST activity is considered as a biomarker for arthritis, asthma, COPD, and multiple forms of cancer, and is thought to substantially contribute to the innate or acquired resistance of specific neoplasms to anticancer therapy.

Assay principle

The ZellX® Glutathione S-Transferase Fluorescent Activity Kit is designed to quantitatively measure the activity of GST present in a variety of samples.

A GST standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. The kit utilizes a non-fluorescent substrate of GST enzyme which covalently binds glutathione (GSH) to yield a highly fluorescent product. In brief, the sample or standard is mixed with the supplied Detection Reagent and GSH, incubated at room temperature (RT) for 30 minutes to generate the fluorescent product which is read at 460 nm with excitation at 390 nm. The activity of GST in the sample is calculated with consideration of the dilution.

General information

Materials supplied in the Kit

Component	*Quantity*
Glutathione S-Transferase Standard (10 U/mL)	50 µL
Glutathione (20 mM)	300 µL
Assay Buffer	45 mL
GST Detection Reagent	1 vial
DMSO	2 mL
Black Half Area 96-Well Plate	1 plate

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Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit. DMSO Can be stored tightly capped at RT as it will freeze at 4° C.

Materials required but not supplied

Phosphate Buffer Saline (PBS)

Microplate reader capable of reading fluorescent at 460 nm with excitation at 390 nm

Centrifuge, Vortex mixer

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

Disposable 1.5-2 mL microtubes for sample preparation

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Dimethyl sulfoxide (DMSO) is a powerful aprotic organic solvent that has been shown to enhance the rate of skin absorption of skin-permeable substances. Wear protective gloves when using the solvent especially when it contains dissolved chemicals. **NOTE: DMSO can dissolve certain plastics, e.g. reservoirs of multichannel/repeater pipettes.**

General remarks

- Equilibrate all kit components to RT 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once to prevent cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

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Assay protocol

Reagent preparation

i. **Glutathione Working Solution:** Prepare a 1:10 dilution of Glutathione with Assay Buffer (1 part Glutathione with 9 parts Assay Buffer), and mix well. Discard any excess Glutathione Working Solution.

ii. **Detection Reagent:** Allow the ziploc bag to warm completely to room temperature prior to opening. Add 300 µL of the provided DMSO to the vial of GST Detection Reagent and vortex thoroughly. Store any unused reconstituted Detection Reagent at 4°C in the ziploc pouch with desiccant and use within **2 weeks**. Prepare a 1:10 dilution of reconstituted Detection Reagent with Assay Buffer (1 part reconstituted Detection Reagent with 9 parts Assay Buffer), and mix well. Discard any excess diluted Detection Reagent.

Sample preparation

After collecting the sample, extraction should be immediately carried out in accordance with related instruction. After extraction, experiment should be conducted immediately, otherwise, keep the sample at -70 or -80˚C. Avoid repeated freeze-thaw cycles.

Samples must be diluted in Assay Buffer. Dilutions should be made to ensure that protein levels for samples fall within the standard curve range.

All samples and standards must be used within 2 hours of dilution.

I. **Serum, Plasma:**

- Collect the fresh serum or plasma with heparin or EDTA.
- Centrifuge at 600 g for 10 min at 4˚C.
- Transfer the serum or plasma from the red blood cells into fresh tubes.
- Samples that are not clear or contain visible particulate should be centrifuged prior to using.
- Serum should be diluted ≥ 1:2 by taking one part of serum and adding 2 or more parts of Assay Buffer prior to conducting assay.

II. **Urine:**

- Collect the urine sample.
- Samples that are not clear or contain visible particulate should be centrifuged prior to using.
- Serum should be diluted ≥ 1:2 by taking one part of urine and adding 2 or more parts of Assay Buffer prior to conducting assay.

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- **Normalize the sample value based on urinary creatinine levels using our Urine Creatinine assay kit Cat NO. ZX-44110-96**.

III. **Cell lysate:**

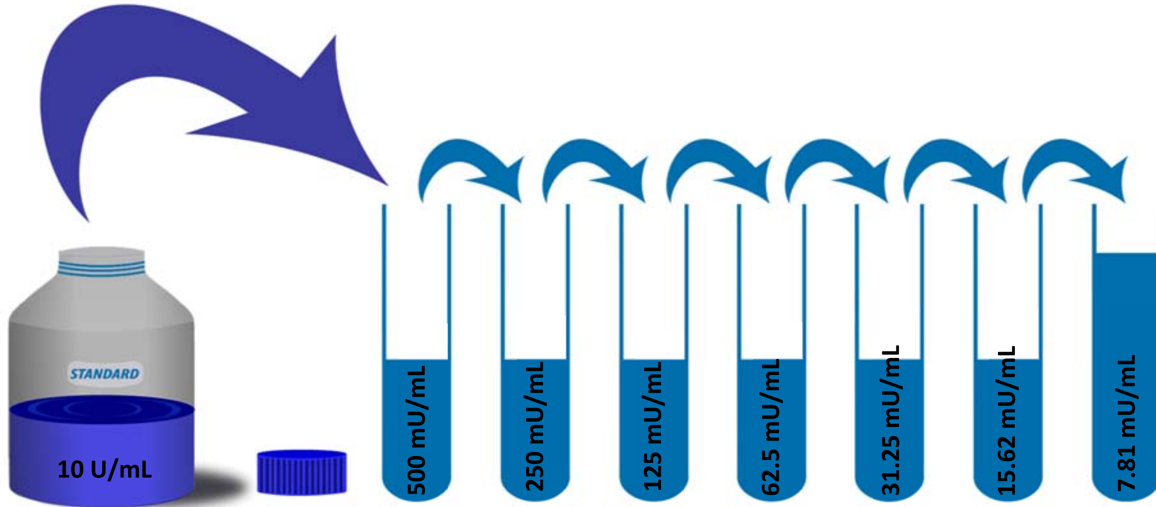
- Centrifuge > 1 x 10^7 cells in suspension at 250 g for 10 minutes at 4°C and Discard the supernatant.
- Homogenize the pellet in 1 mL of cold Assay Buffer per 10^7 cells.
- Lyse cells by vigorous vortexing, freeze/thaw cycling or other suitable disruption method.
- Centrifuge at 10000 g at 4°C for 15 minutes and collect supernatant.
- Sample should be diluted ≥ 1:2 by taking one part of supernatant and adding 2 or more parts of Assay Buffer prior to conducting assay (Based on cell type and number).
- Normalize the sample value based on protein levels.

All the samples must be used within 2 hours of dilution

Standard preparation

- Prepare a 1:20 dilution of GST Standard with Assay Buffer (mix 20 µL of standard with 380 µL of Assay Buffer), and label as the Standard No.7 (500 mU/mL).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 mU/mL standard.

No.	Concentration	Material needed
Standard No.7	500 mU/mL	20 µL GST Standard + 380 µL Assay Buffer
Standard No.6	250 mU/mL	200 µL Standard No.7 + 200 µL Assay Buffer
Standard No.5	125 mU/mL	200 µL Standard No.6 + 200 µL Assay Buffer
Standard No.4	62.5 mU/mL	200 µL Standard No.5 + 200 µL Assay Buffer
Standard No.3	31.25 mU/mL	200 µL Standard No.4 + 200 µL Assay Buffer
Standard No.2	15.62 mU/mL	200 µL Standard No.3 + 200 µL Assay Buffer
Standard No.1	7.81 mU/mL	200 µL Standard No.2 + 200 µL Assay Buffer
Standard No.0	0 mU/mL	200 µL Assay Buffer



All standard must be used within 2 hours of preparation

Assay Procedure

1. Pipette 50 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 50 µL of Assay Buffer as the Zero standard.
3. Add 25 µL of the Detection Reagent to each well using a multichannel/repeater pipet.
4. Add 25 µL of the Glutathione Working Solution to each well using a multichannel/repeater pipette.
5. Gently tap the side of the plate and mix well.
6. Incubate at room temperature for 30 minutes.
7. Read the fluorescent intensity at 460 nm with the excitation at 370-410 nm.

Calculation

- Average the duplicate Fluorescent Unit (FLU) readings for each standard and sample.
- Subtract the mean FLUs for the zero standard from all FLU values
  (for example if the FLU value of zero standard, and standard 6 are 0.087, and 1.086 respectively; then the adjusted FLUs equal 0 and 0.999 respectively.)
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted FLU values
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

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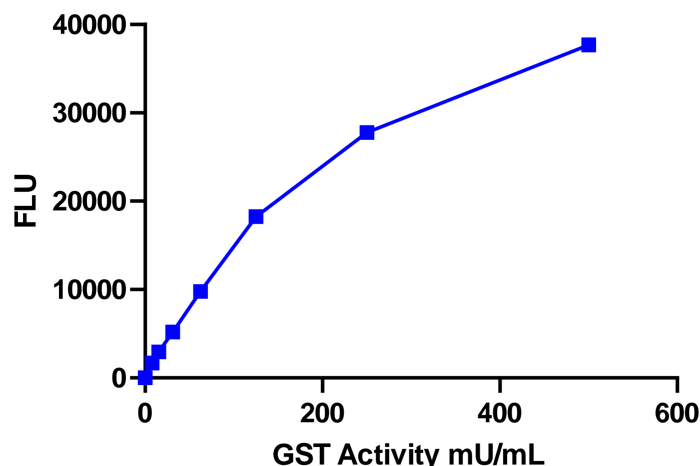

A typical standard curve of ZellX® GST Fluorescent Activity kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® GST assay was determined as 1.90 mU/mL.

Sensitivity

The sensitivity of the ZellX® GST assay was determined as 2.70 mU/mL.

Precision

Intra-Assay Precision (Precision within an assay): 4 serum samples were tested 16 times in an assay.

Inter-Assay Precision (Precision between assays): 4 serum samples were tested in duplicate on 20 different assays over multiple days.

Item	*%CV*
Intra assay	6.6, 4.6, 4.2, 5.6
Inter assay	11.0, 15.9, 12.6, 10.4

Interferences

A variety of solvents and detergents were tested as possible interfering substances in the assay. Approximately 10% change was seen in the GST activity in the presence of 1% methanol or DMSO in the

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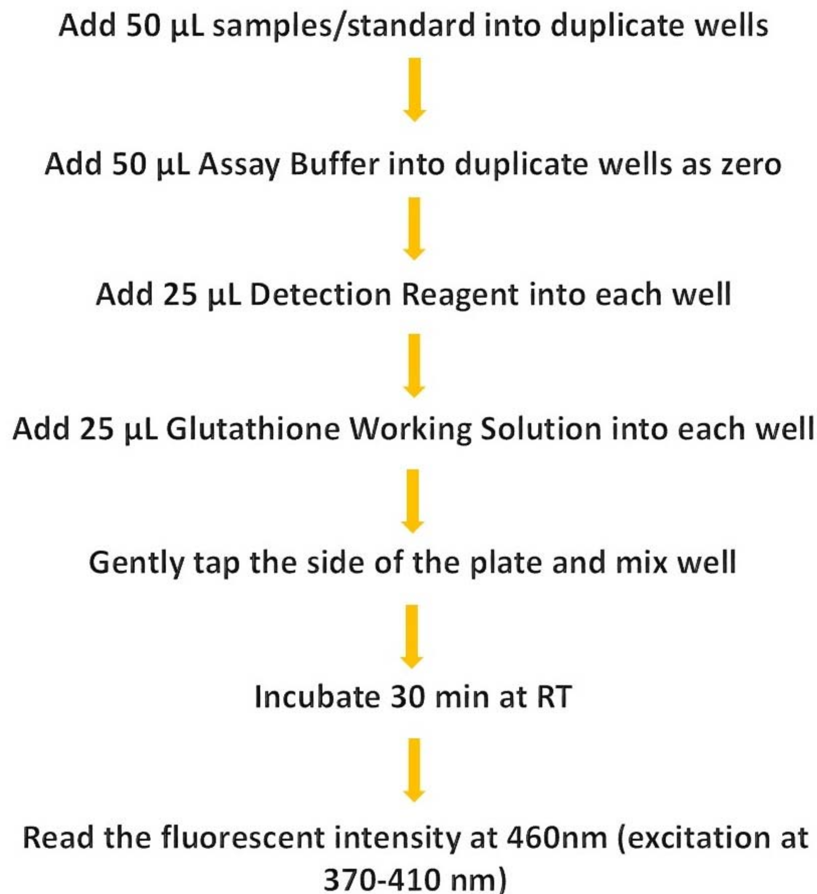
sample. Three detergents were also tested: Triton X-100, Tween 20 and SDS. At 0.01% concentration in the sample both SDS and Tween showed no change in activity, whereas Triton showed > 47% decrease at 0.01%.

Bilirubin levels of 2.5 µg/mL in the sample showed < 5% decrease in GST activity.

Protocol summary



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References

1. Habig, W, et al. “Glutathion S-Transferases: The First Enzymatic Step in Mercapturic Acid Formation” J. Biol. Chem. 1974 249(22):7130-7139.
2. Cook, JA, et al., “Differential Specificity of Monochlorobimane for Isozymes of Human and Rodent Glutathione S-Transferases” Cancer Res. 1991 51:1606-1612.
3. Dalle-Donne, I, et al. “Biomarkers of Oxidative Damage in Human Disease” Clinical Chemistry, 2006 52(4):601-623.
4. Surapneni, KM & VSC Gopan, “Lipid Peroxidation and Antioxidant Status in Patients with Rheumatoid Arthritis”
5. Ind. J. Clin. Biochem. 2008 23(1):41.44.
6. Mohan, SK & O Venkataramana. “Status of Lipid Peroxidation, Glutathione, Ascorbic Acid, Vitamin E and Antioxidant Enzymes in Patients with Osteoarthritis” Ind. J. Med. Sci. 2007 61:9-14.
7. Ferrandina, G., et al., “Glutathione S-Transferase Activity in Epithelial Ovarian Cancer: Association with Response to Chemotherpapy and Disease Outcome” Ann. Oncol. 1997 8:343-350.
8. Otitoju, O & Onwarah, INE. “Glutathione S-transferase (GST) Activity as a Biomarker in Ecological Risk Assess- ment of Pesticide Contaminated Environment” African J. Biotech. 2007 6(12) 1455-1459.
9. Shea, TC, et al., “Identification of an Anionic Form of Glutathione Transferase Present in Many Human Tumors and Human Tumor Cell Lines” Cancer Res. 1988 48:527-533.
10. Shea, TC, et al., “Glutathione Transferase Activity and Isozyme Composition in Primary Human Breast Cancers” Cancer Res. 1990 50:6848-6853.

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