GLUTATHIONE-S-TRANSFERASE

UV Method **25 Tests**
For Research Only

INTRODUCTION :

Glutathione S- transferases (GST) are 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, The glutathione conjugates are metabolized further to mercapturic acid and then excreted . These classes are comprised of both cytosolic and microsomal enzymes .

PRINCIPLE :

The Biodiagnostic 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. 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.

SAMPLE :

Plasma , erythrocyte lysate , cell lysate and tissue homogenate (cytosolic and microsomal) .

REAGENTS :

1.	Phosphate buffer , pH 7.4
2.	Glutathione Reduced (GSH) (Powder)
3.	Chloro, 2,4-dinitrobenzen (CDNB)
4.	Trichloroacetic acid

Preparation of Solution :

- Reconstitute **reagent 2** in 5 ml d. water. When not in use store at - 20°C or below.
- **Reagents 1, 3 and 4** ready for use when not in use store at 4 - 8°C.

STABILITY :

The reagents are stable up to the expiry date specified when stored at the proper temperature indicated
R1, R3 and R4 Store at 4 °C
R2 Store at – 20 °C or below

PROCEDURE:

	Sample ml	Blank ml
Buffer (R1)	**1.0**	**1.0**
Sample	**0.05**	**0.05**
GSH (R2)	**0.1**	**0.1**
	Incubate at 37ºC for 5 min. then add:	
CDNB (R3)	**0.1**	**-**
	Mix well. Incubate at 37ºC for exactly 5 min. Terminate the reaction by adding :	
TCA (R4)	**0.1**	**0.1**
CDNB (R3)	**-**	**0.1**

Mix well, centrifuge at 3000 r. p. m. for 5 min. measure the absorbance of sample (A_{sample}) against the blank at 340 nm.

CALCULATION :

GST Activity :

Plasma (U / L) = A (sample) x 2812

Tissue (U /g tissue) = A (sample) x $\frac{2.812}{\text{g . tissue used}}$

QUALITY CONTROL :

For accuracy and reproducibility control :

Assayed Multi – Sera Normal and Elevated

SAMPLE PREPARATION

The procedure for tissue homogenates and cell lysates will result in assaying total GST activity (cytosolic and microsomal) . To separate the two enzymes, centrifuge the10,000 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 sample at -80°C .

Tissue Homogenate

1. Prior to dissection, perfuse tissue with a PBS (phosphate buffered saline) solution, pH 7.4 . containing 0.16 mg / ml heparin to remove any red blood cells .
2. Homogenize the tissue in 5 – 10 ml cold buffer (i , e , 100 mM potassium phosphate, pH 7.0, containing 2 mM EDTA) per gram tissue.
3. Centrifuge at 4,000 rpm 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 rpm 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 cold buffer (i, e, 100 mM potassium phosphate, pH 7.0, containing 2 mM EDTA) .
3. Centrifuge at 4,000 rpm 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. Centerifuge the blood at 3,000 rpm for 10 minutes at 4 °C . Pipet off 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 and discard .
4. Lyse the erythrocytes (red blood cells) in 4 times its volume of ice – cold HPLC grade water or D.W .
5. Centrifuge at 4,000 rpm for 15 minutes at 4 °C.
6. Collect the supernatant (erythrocyte lysate) for assaying and store on ice. If not assaying on the same day , freeze at - 80°C. The sample will be stable for at least one month.

REFERENCE :

Habig W and Pabst M Jakoby, W. J . Biol . Chem. (1974) 249, 7130 – 7139

BIODIAGNOSTIC
DIAGNOSTIC AND RESEARCH REAGENTS

Glutathione - S- Transferase

UV Method

+4 to +8ºC **25 Tests**

CAT. No.	GT 25 19

FOR RESEARCH USE ONLY

REAGENTS

R1	Buffer	**50 ml**
R2	GSH	**1 vial**
R3	CDNB	**5 ml**
R4	TCA	**5 ml**

CONTACTS

Tele: 02-33385184
Mobil: 0109 – 349 20 77
Fax : 02-33385184 (102)
e.maile : info@bio-diagnostic.com
Website: www.bio-diagnostic.com
Adress: 29 Tahreer St., Dokki, Giza, Egypt