

## RECOMBINANT HUMAN GLUTATHIONE S-TRANSFERASE (GSTs) ENZYMES

Product No.	Description
CYP120	GSTA1
CYP121	GSTM1
CYP122	GSTP1
CYP099	Control cytosol

### PRODUCT DESCRIPTION:

Human Glutathione S-Transferase recombinant enzymes are expressed in E-coli (10 mg/mL) and are supplied in 10 mM sodium phosphate (pH 7.4) with 140 mM NaCl. Each vial of human GST recombinant enzyme contains 1 mg of cytosolic protein.

**STORAGE:** ≤ -80°C

### MATERIALS

200 mM potassium phosphate, pH 6.5  
100 mM reduced glutathione in water  
(prepare fresh and keep on ice)  
Deionized water  
Substrate solution  
1x PBS (for enzyme dilution, if required)  
1 M HCl, methanol or acetonitrile as stop  
reagent

### EQUIPMENT

Water bath set to 37°C  
Suitable polypropylene tubes  
Centrifuge

### INCUBATION PROCEDURE:

Incubations are usually conducted in 100 mM potassium phosphate buffer, pH 6.5, although other buffers may be used.

### DRUG METABOLISM

- 1) Thaw the GST on ice and keep on ice once thawed. Mix gently just before use.
- 2) Prepare incubations on ice, using the guide below. Reactions are initiated by the addition of the cofactor, reduced glutathione. Multiple incubations should be prepared from a pre-mix (see below).

**For a single 0.2 ml incubation:**

200 mM potassium phosphate pH 7.4	100 µl
Water	(98 – x – y) µl
Substrate / test compound	x µl
GST	y µl

**Pre-mix for 20 x 0.2 ml incubations:**

200 mM potassium phosphate pH 7.4	2000 µl
Water	(1960 – xx – yy) µl
Substrate / test compound	xx µl
GST	yy µl

The volume of substrate will be determined by the required final concentration. Solvent concentration (e.g. methanol, DMSO) should be kept to a minimum with a maximum concentration in the assay of 1% (v/v).

The concentration of GST will be dependent on the requirements of the assay and the activity of the enzyme with the substrate being used. Typical protein concentrations can be found on the data sheet accompanying the specific GST product being used. It should be borne in mind, however, that these concentrations are specific to the substrate being used and are set to minimise substrate loss (less than 10% across the assay). If you are looking for substrate loss in the assay then the concentration of GST should be increased accordingly.

- 3) Add the appropriate volume of pre-mix to each assay tube (1.5 ml polypropylene microtubes work well) and pre-incubate at 37°C for 5 min. The assay volume can be adjusted as required: we also use 1 ml final volume assays in 15 ml polypropylene conical tubes.
- 4) Initiate the reaction by adding 2 µl of 100 mM reduced glutathione (final concentration 1 mM) to each tube, and incubate at 37°C (typically 5 – 30 minutes, but this depends on the GST and substrate being used). Higher final concentrations of reduced glutathione may result in higher enzyme activity.
- 5) Stop the reaction(s) by the addition of one of the following:
  - 0.1 volumes 1 M HCl (20 µl for a 200 µl incubation)
  - 0.5 volumes acetonitrile (100 µl for a 200 µl incubation)
  - 1 volume methanol (200 µl for a 200 µl incubation)
- 6) Place the samples on ice for at least 10 minutes and then centrifuge: approximately 13,000 rpm for 10 mins for microtubes or 4,000 rpm for 20 mins for 15 ml tubes)
- 7) Recover the supernatants for further analysis.

**CAUTION:**

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**North America & Asia Pacific** 📞 516-483-1196  
@ customerservice@bioivt.com  
PO Box 770, Hicksville NY 11802-0770, U.S.A.

**Europe, Middle East & Africa** 📞 44 1444 707333  
@ cseurope@bioivt.com  
West Sussex RH15 9TN, U.K.

🌐 [BioIVT.com](http://BioIVT.com)