Glutathione S-transferase activity assay

*From Sigma, with modifications by JP Béguel*

Reagents:

* Tampon de lyse
* DC protein assay (Bio-Rad) or other protein concentration assay
* Sigma GST assay kit: Dulbecco’s PBS, sample buffer, CDNB (1-chloro-2,4-dinitrobenzene, substrate), L-glutathione reduced, GST (control)
* Ultrapure water

Equipment:

* Materials for grinding tissue
* Tissue homogenizer
* Chilled centrifuge
* Microplates
* Plate reader

Sample preparation

Follow extraction protocol using tampon de lyse

[*protocol from Beguél et al. 2013*

*Cryogenically grind frozen gill samples. Use 75 mg of gill powder for extraction for GST enzymatic assay.*

*Homogenize 75 mg of gill tissue in 250 µl extraction buffer (phosphate buffered saline solution, EDTA 1 mM, Triton X-100 0.1%) with Ultra-Turrax.*

*Centrifuge at 15,000xg for 10 min, 4°C*

*Collect supernatant and store at -80°C*]

Protein concentration determined using DC protein assay (Bio-Rad)

GST assay

Before running samples, make a standard curve using different dilutions to determine correct dilution.

* JPB used undiluted
* Sigma kit recommends 10x dilution

Dilute GST standard (2 µl GST + 18 µl tampon de lyse) – kit recommends using sample buffer, but want standard to be similar to samples. Scale up to test different concentrations if using tampon de lyse.

Prepare L-glutathione: Dissolve 246 mg of L-glutathione in ultrapure water to a final volume of 4 mL. Keep solution on ice and use it the day it is prepared. (The solution can be stable for several months when aliquoted and stored at -20°C.)

Thaw kit components on ice.

Warm Dulbecco’s PBS and CDNB solution to 25°C.

A 10 mL reaction master mix is sufficient for 50 assays in a 96-well plate. The solution must be used within 60 minutes of preparation.

**Recipe for reaction master mix**

|  |  |  |
| --- | --- | --- |
| Reagent | Vol for 10 reactions | Vol for 1 reaction |
| Dulbecco’s PBS | 9.8 ml | 980 µl |
| 200 mM L-glutathione reduced | 0.1 ml | 10 µl |
| 100 mM CDNB | 0.1 ml | 10 µl |

*Addition of CDNB may make solution cloudy, but cloudiness goes away once mixed.*

Each well should have a final volume of 200 µl reaction. For blanks, use 200 µl reaction master mix. For control, 196 µl master mix + 4 µl GST. For sample, 1-20 µl of sample + 190 µl master mix (check sample volume using a standard curve, JPB used 10 µl). All reactions should be run in triplicate. [*Determine control (GST) and sample concentration prior to running full assay.*]

Mix plate gently for a few seconds to mix well.

Read the absorbance at 340 nm immediately after reaction preparation every 41s for 7 minutes.