Assay Procedure

All samples can be stored at 4°C. Remove supernatant for assay.

enzymes; rather use a rubber policeman. Homogenize or sonicate cells in Assay Buffer.

Cell Lysate: collect cells (~4 millions cells) by centrifugation at 2,000×g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 100 mM potassium phosphate (pH 7.0) and 2 mM EDTA. Centrifuge at 10,000×g for 15 min at 4°C. Remove supernatant for assay.

Sample Preparation:

Tissue: prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) with a Dounce homogenizer in ~250 µL cold 100 mM potassium phosphate, pH 7.0 containing 2 mM EDTA. Freeze the homogenized tissue at ~80°C to lyse the cells. After freezing, thaw and centrifuge samples at 10,000×g for 15 minutes at 4°C. Remove supernatant for assay.

Cell Lysate: collect cells (~4 millions cells) by centrifugation at 2,000×g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 100 mM potassium phosphate (pH 7.0) and 2 mM EDTA. Centrifuge at 10,000×g for 15 min at 4°C. Remove supernatant for assay.

All samples can be stored at ~20 to ~80°C for at least one month.

Assay Procedure:

1. Reagent Preparation. Bring all reagents to the desired reaction temperature (e.g. 25°C) prior to assay. Briefly centrifuge tubes before use.

2. Transfer 20 µL of each sample into separate wells.

3. Prepare enough Working Reagent (WR) for all reaction wells by mixing, for each 96-well assay, 184 µL Assay Buffer, 1 µL Reconstituted Glutathione, and 1 µL CDNB.

4. Add 180 µL WR to all samples and tap plate briefly to mix.

5. Read OD_{405nm} at time 0 min and at least four other time points between 0 min and 10 min. If available we recommend reading the plate in a plate reader capable of kinetic measurements and set it to read the OD_{405nm} every min for 10 min.

**CALCULATION**

Plot the OD_{405nm} versus time and use OD values in the linear part to determine the GST activity in a sample, which is computed as follows:

$$\text{GST Activity} = \frac{\frac{\text{OD}_{405} - \text{OD}_{405}}{t}}{\frac{0.0096 \text{ µM}^{-1} \cdot \text{cm}^{-1} \cdot \text{l}}{\text{OD}_{405} - \text{OD}_{405}}} \times \frac{1}{t} \times \frac{V_{\text{Total}}}{V_{\text{Sample}}} \times n$$

where OD_{405} and OD_{405} are OD’s at two different time points in the linear range of the curve and t is the time difference between the two time points. For example, if measurements at t = 0 and t = 10 min are used, then in the equation OD_{405} is the OD at 0 min, OD_{405} is the OD at 10 min.

**MATERIALS REQUIRED, BUT NOT PROVIDED**

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.

**REFERENCES**

