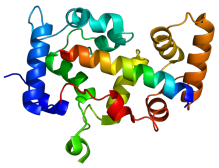
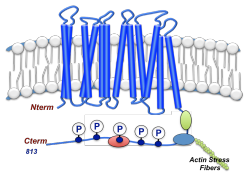


Enzyme Assay for Glutathione S-Transferase Protocol



INTRODUCTION

Glutathione S Transferase (GST) is an enzyme involved in detoxification of a wide range of compounds and is involved in reducing free radical damage in red blood cells. The enzyme is easily purified by affinity chromatography and has been used as a fusion partner for many recombinant proteins. Identification of GST is done by western blotting or more easily by enzymatic assay.

Enzyme Reaction: Glutathione –SH + CDNB -> Glutathione –S-CDNB

The reaction is measured by observing the conjugation of 1-chloro, 2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH). This is done by watching an increase in absorbance at 340nm. One unit of enzyme will conjugate 10.0 nmol of CDNB with reduced glutathione per minute at 25°C.

PROTOCOL

1. **Stock Solutions**
   GSH is prepared in ethanol and can be stored at –20°C for one month. CDNB can be frozen/thawed for no more than five times. Allow all powders to come to room temperature prior to measuring to reduce condensation of solids.

   Assay Buffer
   - 100 mM CDNB dissolved in Ethanol and stored in microfuge tubes
   - 100 mM Reduced Glutatione
   - Assay buffer – PBS adjusted to pH = 6.5

   Assay Cocktail
   - 980 µl PBS pH 6.5
   - 10 µl of 100 mM CDNB
   - 10 µl of 100 mM Glutatione
   - Mix – the solution may be cloudy at first, but should clear up after mixing.

2. **Assay**
   - For each sample and a blank, place 900 µl of enzyme cocktail into 1.5 ml plastic cuvettes.
   - Incubate at 30°C in spectrophotometer for 5 min.
   - To the blank cuvette add 100 µl PBS and zero spec.
   - Add 100 µl of sample to cuvettes and mix.
   - Measure absorbance at 340 nm for five min.

3. **Calculations**
   - Determine the rate where the reaction is linear – this is the λ_{340}/min.
   - Subtract the λ_{340}/min for the blank reaction from the λ_{340}/min for each sample reaction
   - The molar extinction of CDNB is 0.0096 μM^{-1}/cm.
     GST activity = [(Adjusted λ_{340}/min)/ 0. 0096 μM^{-1}/cm] x (1.0 ml /0.1 ml) x any sample dilution
     = U/ml

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