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Glutathione Reductase (GR) Assay Kit (Cat #: E-144)

COMPONENTS: GR Assay Solution: 40 ml, store at 4°C (for 100 assays)
NADPH (50x): 9 mg, **store in small aliquots at -80ºC after reconstitution in 0.9 ml NADPH Buffer**
NADPH Buffer: 1 ml, store at 4°C
GR Substrate: 120 mg, store at 4ºC
10x Cell Lysis Solution: 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The GR activity assay kit uses oxidized glutathione as substrate and is based on coupled oxidation of NADPH, which exhibits a decreased absorbance in 340 nm (extinction coefficient = 6.22 $mM^{-1}cm^{-1}$). The enzyme assay requires a UV-transparent 96-well plate or 0.2-ml quartz cuvette for measuring changes in $O.D._{340\ nm}$. The kit is stable for at least one year if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up at least ~10^5 washed cells in 50–100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~5 mg tissue in 0.5 ml).
2. Centrifuge lysate in a microfuge at 4ºC at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range for the assay is 0.2 – 2 mg/ml.

NADPH reconstitution: Spin NADPH vial briefly to deposit contents. Add 0.9 ml NADPH Buffer to the vial and dissolve NADPH by vortexing. Store solution in small aliquots shielded from light at -80°C. Avoid multiple freeze-thaw cycles. *Do not over thaw NADPH solution.*

Solution preparation

Dim room light for the assay. Prepare fresh solution for each assay. Calculate the amount of control solution and reaction solution required for each assay. Each sample requires 0.2 ml control solution and 0.2 ml reaction solution. Scale up solution preparation as needed. An example for preparation of 1 ml control solution and 1 ml reaction solution is as follows:

2 ml working solution = 2 ml GR Assay Solution + 40 µl NADPH

1 ml control solution = 1 ml working solution

1 ml reaction solution = 1 ml working solution + 2.5 mg GR Substrate (dissolve substrate by vortexing)

Enzyme assay:

1. Use a UV-transparent 96-well plate (or 0.2-ml quartz cuvette) for the assay. Set wavelength at 340 nm for measurement of $O.D._{340\ nm}$.
2. Pipet 20 µl of each sample to duplicate wells of the plate (for control set and reaction set).
3. Add 0.2 ml control solution to each control well and 0.2 ml reaction solution to each reaction well. Agitate plate for 10 sec to mix contents. Incubate plate at 37°C shielded from light for 30 min.
4. Read $O.D._{340\ nm}$. GR activity will cause a decreased $O.D._{340\ nm}$ reading. Subtract the reaction well reading from the control well reading for each sample. Use the subtracted optical reading (**ΔO.D.**) to calculate GR enzyme activity as described below.
5. GR activity in IU/L = µmol/(L•min) = **ΔO.D.** x 1000 x 220 µl / (30 min x 1 cm x 6.22 x 20 µl) = **ΔO.D. x 58.95**. Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (cat# E-101). Lysate protein concentration should be increased if **ΔO.D.** is near 0.

Additional information

10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.