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Glucose-6-phosphatase (G6Pase) Assay Kit (Cat #: E-120)

COMPONENTS: G6Pase Assay Solution- 30 ml (150 wells), store at 4ºC (**swirl solution briefly prior to pipetting**)
10x Lysis Buffer- 25 ml, store at 4ºC (dilute 10-fold with ice-cold dH_2O)
10x Extraction Buffer- 20 ml, store at 4ºC (dilute 10-fold with ice-cold dH_2O)
G6Pase Control- 1 ml, store at 4ºC
G6Pase Reaction- 0.4 ml, store at -20ºC **after reconstitution**
TCA Solution (10%)- 4 ml, store at 4ºC (**caution: avoid skin contact**)

Preparation of cell/tissue lysates:

1. **Cells**: Bring up at least ~10^6 freeze-thawed cells in ~0.1 ml ice-cold 1x Lysis Buffer by pipetting up and down. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Collect supernatant for assays such as Hexokinase, GAPDH, and LDH. Resuspend pellet (membrane fraction) in ~50 µl ice-cold 1x Extraction Buffer by pipetting up and down thoroughly, making sure the membrane fraction is homogenized. Leave homogenate on ice for ~10 min with agitation. Repeat homogenization. Briefly centrifuge homogenate at ~5,000 rpm for 1 min. Harvest supernatant for G6Pase assay. Recommended protein concentration is 0.5 - 2 mg/ml (use more cells for sample preparation if protein concentration is too low).
2. **Tissue:** Mechanically homogenize freeze-thawed tissue in ice-cold 1x Lysis Buffer (~5 mg tissue in 0.5 ml). Centrifuge homogenate in a cold microfuge at ~14,000 rpm for 5 min. Collect supernatant for assays such as Hexokinase, GAPDH, and LDH. Resuspend pellet (membrane fraction) in ~0.3 ml ice-cold 1x Extraction Buffer by pipetting up and down thoroughly, making sure the membrane fraction is homogenized. Leave homogenate on ice for ~10 min with agitation. Repeat homogenization. Briefly centrifuge homogenate at ~5,000 rpm for 1 min. Harvest supernatant for G6Pase assay.

Reconstitution of G6Pase Reaction: Tap G6Pase Reaction vial briefly to deposit contents prior to opening. Add 0.4 ml G6Pase Control to the G6Pase Reaction vial. Vortex tube to dissolve contents. The reconstituted G6Pase Reaction solution should be stored at -20°C. *The solution may exhibit precipitates upon prolonged freezing. Vortex solution to re-dissolve contents prior to pipetting if necessary.*

Enzyme assay: Assay is initially set up in 0.5-ml microtubes and is completed in a plain (uncoated) 96-well plate.

1. Keep thawed G6Pase Control and G6Pase Reaction on ice. *Do not over thaw.* Add 2.5 µl G6Pase Control to each tube of the control set and 2.5 µl G6Pase Reaction to each tube of the reaction set. Keep all tubes on ice.
2. First add 20 µl 1x Extraction Buffer to a control tube (as background control) and a reaction tube (as background reaction). Close tubes.
3. Then add 20 µl of each sample to a sample control tube and a sample reaction tube. Mix contents by pipetting up and down. *Do not vortex.* Close tubes.
4. Transfer all control and reaction tubes to a 37°C incubator and incubate for 10 min.
5. Transfer tubes back to ice after 10 min. Add 22.5 µl ice-cold TCA Solution to each control tube and reaction tube. Vortex tubes immediately and vigorously. Allow tubes to sit on ice for 10 min.
6. Centrifuge tubes in a microfuge at ~14,000 rpm for 5 min. Transfer 30 µl supernatant from each control tube and each reaction tube to a 96-well plate.
7. Swirl G6Pase Assay solution briefly to disperse precipitates prior to pipetting. Add 0.2 ml to each control well and reaction well. Briefly agitate plate to mix. Allow plate to sit on bench for 5 min.
8. Measure **O.D.**$_{630\ nm}$ using a plate reader. Subtract control well reading from reaction well reading to generate **ΔO.D.** for the background (**ΔO.D.** $_{background}$) and each sample (**ΔO.D.** $_{sample}$).
9. Sample G6Pase activity = **ΔO.D.** $_{sample}$ - **ΔO.D.** $_{background}$. Enzyme activity may be normalized by sample protein concentration or GAPDH activity (see #E-101).

Additional information:

G6Pase Assay Solution contains hydrochloric acid. Both hydrochloric acid and trichloroacetic acid (TCA) are highly corrosive. Use caution in handling the solutions. Please visit the product webpage or contact us for SDS information.