



TECHNICAL MANUAL

Glucose-6-phosphatase (G6Pase) Activity Assay Kit

- **SKU CODE:** BR00008
- **SIZE:** 150 wells
- **DETECTION PRINCIPLE:** Colorimetric
- **RUO:** Research-Use-Only

Glucose-6-phosphatase (G6Pase) Activity Assay Kit

Please read entire manual carefully before starting experiment.

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1. Key Features

Specification:

150 wells

Measuring instrument:

Microplate reader (O.D._{630 nm})

Sample type:

Cell/tissue lysates (membrane fraction)

2. Storage & Expiry

This product should be stored according to the conditions listed for each kit component. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

3. Product Description

The Glucose-6-phosphatase (G6Pase) Activity Assay Kit allows for measurement of G6Pase activity in the membrane fraction of cell and tissue lysates. The assay is initially set up in 0.5-ml microtubes and is completed in a plain (uncoated) 96-well plate, with absorbance measured at 630 nm.

4. Kit Contents

No	Component Name	Size	Storage
1	G6Pase Assay Solution	30 ml (150 wells)	4°C (swirl solution briefly prior to pipetting)
2	10x Lysis Buffer	25 ml	4°C (dilute 10-fold with ice-cold dH ₂ O)
3	10x Extraction Buffer	20 ml	4°C (dilute 10-fold with ice-cold dH ₂ O)
4	G6Pase Control	1 ml	4°C
5	G6Pase Reaction	0.4 ml	-20°C after reconstitution
6	TCA Solution (10%)	4 ml	4°C (caution: avoid skin contact)

Note: All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.

Additional materials required:

1. 37°C incubator.
2. Microplate Reader (630 nm).
3. Plain (uncoated) 96-well plate.
4. 0.5-ml microtubes.
5. Refrigerated microcentrifuge (~14,000 rpm), vortex mixer and tissue homogenizer.
6. Precision pipettes and disposable pipette tips. Distilled water.

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We

advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

7. 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 Assay Genie for SDS information.

6. Reagent Preparation

1. **1x Lysis Buffer and 1x Extraction Buffer:** Dilute 10x Lysis Buffer and 10x Extraction Buffer 10-fold with ice-cold dH₂O.
2. **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.

7. Sample Preparation

Cells

1. Bring up at least ~10⁶ freeze-thawed cells in ~0.1 ml ice-cold 1x Lysis Buffer by pipetting up and down.
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Collect supernatant for assays such as Hexokinase, GAPDH, and LDH.
3. 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.
4. 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).

Tissue

1. Mechanically homogenize freeze-thawed tissue in ice-cold 1x Lysis Buffer (~5 mg tissue in 0.5 ml).
2. Centrifuge homogenate in a cold microfuge at ~14,000 rpm for 5 min. Collect supernatant for assays such as Hexokinase, GAPDH, and LDH.
3. 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.
4. Briefly centrifuge homogenate at ~5,000 rpm for 1 min. Harvest supernatant for G6Pase assay.

8. Assay Procedure

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

1. **Prepare tubes:** Keep thawed G6Pase Control and G6Pase Reaction on ice. Do not over thaw. Add 2.5 μ l G6Pase Control to each tube of the control set and 2.5 μ l G6Pase Reaction to each tube of the reaction set. Keep all tubes on ice.
2. **Background tubes:** First add 20 μ l 1x Extraction Buffer to a control tube (as background control) and a reaction tube (as background reaction). Close tubes.
3. **Add samples:** Then add 20 μ l 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. **Incubate:** Transfer all control and reaction tubes to a 37°C incubator and incubate for 10 min.
5. **Stop reaction:** Transfer tubes back to ice after 10 min. Add 22.5 μ l 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 and transfer:** Centrifuge tubes in a microfuge at ~14,000 rpm for 5 min. Transfer 30 μ l supernatant from each control tube and each reaction tube to a 96-well plate.
7. **Color development:** 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:** Measure O.D._{630 nm} using a plate reader. Subtract control well reading from reaction well reading to generate Δ O.D. for the background (Δ O.D. background) and each sample (Δ O.D. sample).

9. Data Analysis

Calculation:

Sample G6Pase activity = **Δ O.D. sample – Δ O.D. background**

Enzyme activity may be normalized by sample protein concentration or GAPDH activity.

Note:

Δ O.D. sample: sample reaction well reading minus sample control well reading at 630 nm.

Δ O.D. background: background reaction well reading minus background control well reading at 630 nm.

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