**Product Information**

**Glycerol 3-Phosphate Dehydrogenase Activity Colorimetric Assay Kit**

Catalog Number **MAK208**  
Storage Temperature –20 °C

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**TECHNICAL BULLETIN**

**Product Description**

Glycerol 3-phosphate dehydrogenase (GPDH; EC 1.1.1.8) catalyzes the reversible conversion of dihydroxyacetone phosphate and NADH to glycerol 3-phosphate and NAD^+_. It forms a glycerol phosphate shuttle and facilitates the transfer of reduced equivalents across the mitochondria to the cytosol. GPDH is important for both carbohydrate and lipid metabolism.\(^1,2\) Mutations in GPDH gene result in transient infantile hypertriglyceridemia.\(^3\)

The Glycerol 3-Phosphate Dehydrogenase Activity Colorimetric Assay Kit provides a simple and sensitive procedure for measuring GPDH activity in a variety of tissues and cells. GPDH activity is determined by measuring a colorimetric product with absorbance at 450 nm (\(A_{450}\)) proportional to the enzymatic activity present. One unit of GPDH is the amount of enzyme required to generate 1.0 \(\mu\)mole of NADH per minute at pH 8 at 37 °C.

**Components**

The kit is sufficient for 100 assays in 96 well plates.

- **GPDH Assay Buffer**  
  Catalog Number **MAK208A**  
  27 mL

- **GPDH Substrate**  
  Catalog Number **MAK208B**  
  1 vl

- **GPDH Probe**  
  Catalog Number **MAK208C**  
  1 vl

- **NADH Standard**  
  Catalog Number **MAK208D**  
  1 vl

- **GPDH Positive Control**  
  Catalog Number **MAK208E**  
  1 vl

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**Reagents and Equipment Required but Not Provided**

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

**Preparation Instructions**

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

- **GPDH Assay Buffer** – Warm to room temperature before use.
- **GPDH Substrate** – Reconstitute with 220 \(\mu\)L of GPDH Assay Buffer. Store at –20 °C. Keep on ice during use. Use within 2 months.
- **GPDH Probe** – Reconstitute with 220 \(\mu\)L of water. Mix well by pipetting. Store at –20 °C. Use within 2 months.
- **NADH Standard** – Reconstitute with 100 \(\mu\)L of GPDH Assay Buffer to generate a 5 mM NADH Standard Solution. Aliquot and store at –20 °C. Keep on ice during use. Use within 2 months.
- **GPDH Positive Control** – Reconstitute with 100 \(\mu\)L of GPDH Assay Buffer. Mix well by pipetting. Aliquot and store at –20 °C.

**Storage/Stability**

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.
Procedure
All samples and standards should be run in duplicate.

NADH Standards
Dilute 20 µL of the 5 mM (5 nmole/µL) NADH Standard Solution with 80 µL of GPDH Assay Buffer and mix well to prepare a 1 mM (1 nmole/µL) NADH Standard Solution. Add 0, 2.5, 5, 7.5, 10 and 12.5 µL of the 1 mM (1 nmole/µL) NADH Standard Solution into a 96 well plate, generating 0 (blank), 2.5, 5.0, 7.5, 10, and 12.5 nmole/well standards. Add GPDH Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue samples (10 mg) or cells (1 × 10⁶) can be homogenized in 200 µL of ice cold GPDH Assay Buffer. Keep on ice for 10 minutes. Centrifuge the samples at 12,000 × g for 5 minutes to remove insoluble material. Transfer supernatant to a fresh tube.

Add 1–50 µL of the samples into duplicate wells. Bring samples to a final volume of 50 µL using GPDH Assay Buffer.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

For samples exhibiting significant background, especially background caused by NADH in the sample, include a Sample Blank for each sample by omitting the GPDH Substrate. The Sample Blank readings can then be subtracted from the sample readings.

For a positive control (optional), add 1–10 µL of the GPDH Positive Control solution to the desired wells. Adjust the final volume to 50 µL with the GPDH Assay Buffer.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of Reaction Mix is required for each reaction (well).

Table 1.
Reagent Mixes

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Standards, Controls, and Samples</th>
<th>Sample Blank</th>
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<tr>
<td>GPDH Assay Buffer</td>
<td>46 µL</td>
<td>48 µL</td>
</tr>
<tr>
<td>GPDH Probe</td>
<td>2 µL</td>
<td>2 µL</td>
</tr>
<tr>
<td>GPDH Substrate</td>
<td>2 µL</td>
<td>–</td>
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</table>

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Measure the absorbance (A₄₅₀) in a microplate reader in kinetic mode for 20–60 minutes at 37 °C. Protect the plate from light during the incubation. It is recommended to take absorbance readings every minute.

Note: Incubation time depends on the activity of GPDH in the samples.

4. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (12.5 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

Note: The NADH Standards can be read at the end of the incubation time.
**Results**

**Calculations**
Plot the absorbance ($A_{450}$) for each well versus time.

Choose two time points (T1 and T2) in the linear range of the plot and determine the $A_{450}$ at each time (ABS1 and ABS2).

**Note:** It is essential that ABS1 and ABS2 fall within the linear range of the standard curve.

Correct for the background by subtracting the measurement obtained for the blank NADH Standard from that of the standards, controls, and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate NADH Standards to plot a standard curve.

**Note:** A new standard curve must be set up each time the assay is run.

Calculate the change in absorbance from T1 to T2 for the samples.

$$\Delta{ABS} = {ABS2} - {ABS1}$$

Subtract the Sample Blank $\triangle{ABS}$ value from the Sample $\Delta{ABS}$ reading to obtain the corrected measurement. Using the corrected measurement, determine the amount of NADH (nmole/well) generated by the GPDH assay between T1 and T2 ($S_a$).

**GPDH activity:**

$$\text{GPDH Activity} = \frac{S_a}{(\text{Reaction Time}) \times S_v}$$

where:

- $S_a = \text{Amount of NADH (nmole) generated in unknown sample well between T1 and T2 from standard curve}$
- Reaction Time = T2 – T1 (minutes)
- $S_v = \text{sample volume (mL) added to well}$

GPDH activity is reported as

$$\text{n mole/min/mL} = \text{milliunit/mL}$$

One unit of GPDH is the amount of enzyme that generates 1.0 µmole of NADH per minute at pH 8 at 37 °C.

**Sample Calculation:**

Amount of NADH ($S_a$) = 5.84 nmole
(from standard curve)

(T1) = 3 minutes
(T2) = 32 minutes

Sample volume ($S_v$) = 0.050 mL

GPDH activity in sample well:

$$\text{nmole/min/mL} = \frac{5.84 \text{ nmole/well}}{(32 \text{ min} - 3 \text{ min}) \times 0.050 \text{ mL/well}} = 4.03$$

One unit of GPDH is the amount of enzyme required to generate 1.0 µmole of NADH per minute at pH 8.0 at 37 °C.

**References**

## Troubleshooting Guide

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<td>Cold assay buffer</td>
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<td>Omission of step in procedure</td>
<td>Refer and follow Technical Bulletin precisely</td>
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<td>Plate reader at incorrect wavelength</td>
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<td>Repeat the sample homogenization, increasing the length and extent of homogenization step</td>
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<td>Aliquot and freeze samples if needed to use multiple times</td>
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<td><strong>Lower/higher readings in samples and standards</strong></td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
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<td>Use of expired kit or improperly stored reagents</td>
<td>Check the expiration date and store the components appropriately</td>
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<td>Allowing the reagents to sit for extended times on ice</td>
<td>Prepare fresh Reaction Mixes before each use</td>
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<td>Refer to Technical Bulletin and verify correct incubation times and temperatures</td>
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<td>Incorrect volumes used</td>
<td>Use calibrated pipettes and aliquot correctly</td>
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<td><strong>Non-linear standard curve</strong></td>
<td>Use of partially thawed components</td>
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<td>Pipetting errors in preparation of standards</td>
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<td>Pipette gently against the wall of the plate well</td>
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<td>Check the equipment and filter settings</td>
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<td>Samples contain interfering substances</td>
<td>If possible, dilute sample further</td>
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<td></td>
<td>Sample readings above/below the linear range</td>
<td>Concentrate or dilute samples so readings are in the linear range</td>
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