Technical Bulletin

Glycogen Assay Kit

Catalogue number MAK016

Product Description

Glycogen is a branched polymer of glucose that serves as the primary short-term energy storage molecule in animals. Glycogen is primarily synthesized in liver and muscle tissue where it can constitute up to 10% of the weight of liver and 1–2% of the weight of muscle tissue. While muscle glycogen is generally utilized locally, liver glycogen serves as an important buffer to regulate blood glucose levels. Glycogen metabolism is dysregulated in diabetes and the glycogen storage diseases due to inborn errors of metabolism.

Glycogen concentration is determined by a coupled enzyme assay, which produces a colorimetric (570 nm)/ fluorometric (λ_{ex} = 535 λ_{em} = 587 nm) product, proportional to the glycogen present.

Components

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

- Hydrolysis Buffer 25 mL
  Catalogue Number MAK016A
- Development Buffer 25 mL
  Catalogue Number MAK016B
- Fluorescent Peroxidase Substrate in DMSO 0.2 mL
  Catalogue Number MAK016C
- Hydrolysis Enzyme Mix 1 vl
  Catalogue Number MAK016D
- Development Enzyme Mix 1 vl
  Catalogue Number MAK016E
- Glycogen Standard, 2 mg/mL 0.1 mL
  Catalogue Number MAK016F

Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Fluorescence or spectrophotometric multi-well plate reader
- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.

Precautions and Disclaimer

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.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C protected from light, is recommended.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Reagent Preparation

Hydrolysis Buffer and Development Buffer – Allow buffers to come to room temperature before use.

Fluorescent Peroxidase Substrate – Thaw the solution at room temperature prior to use. Store protected from light and moisture at -20 °C. Upon thawing, the Fluorescent Peroxidase Substrate is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Fluorescent Peroxidase Substrate 5 to 10-fold with Development Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Hydrolysis Enzyme Mix – Reconstitute in 220 µL of Hydrolysis Buffer. Mix well by pipetting (do not vortex) and keep on ice while in use. Aliquot and store at -20 °C. Use within 2 months of reconstitution.

Development Enzyme Mix – Reconstitute in 220 µL of Development Buffer. Mix well by pipetting (do not vortex) and keep on ice while in use. Aliquot and store at - 20 °C. Use within 2 months of reconstitution.

Procedure

All Samples and Standards should be run in duplicate. Use ultrapure water for the preparation of Samples and Standards.

Glycogen Standards for Colorimetric Detection

Dilute 10 µL of the 2.0 mg/mL Glycogen Standard with 90 µL of ultrapure water to prepare a 0.2 mg/mL Standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.2 mg/mL standard solution into a 96 well plate, generating 0 (assay blank), 0.4, 0.8, 1.2, 1.6, and 2.0 µg/well Standards. Add Hydrolysis Buffer to each well to bring the volume to 50 µL.

Glycogen Standards for Fluorometric Detection

Clear and slightly colored Samples can be assayed directly. Biological fluid samples (e.g., urine and serum) can be assayed directly after centrifuging to remove any particulates.

It is prudent to test several dilutions to determine an optimal dilution factor (n). Appropriate dilution in ultrapure water may be required.

Transfer 50 µL of each Sample in duplicate into separate wells (one well as "Sample" and one well as "Sample Blank")

Sample Preparation

There are a variety of methods for the extraction of glycogen from tissues depending upon the tissue type. Provided below are general methods that can be used, but it is highly recommended to consult the literature regarding isolation of glycogen from specific tissue types. Liquid Samples may be assayed directly.

Tissue (10 mg) or cells (1×10^6) can be homogenized in 200 µL of water on ice. Boil homogenates for 10 minutes to inactivate enzymes. Centrifuge the Samples at 18,000 × g for 10 minutes to remove insoluble material. The supernatant is ready for assay. Add 2-50 µL Samples to a 96-well plate. Adjust volume to 50 µL/well with Hydrolysis Buffer.

For unknown Samples, it is suggested to test several Sample dilutions to ensure the readings are within the linear range of the Standard curve.

Note: Glycogen can be metabolized very rapidly in some tissues following tissue isolation. To minimize glycogen loss during sample preparation, Samples may be flash frozen in liquid nitrogen. Keeping Samples cold during preparation may also decrease glycogen loss in susceptible Samples.

1. Add 2 µL of the Hydrolysis Enzyme Mix to colorimetric assays and 1 µL to fluorometric assays, mix well, and incubate for 30 minutes at room temperature.

   Note: Glucose in the Samples will generate a background signal. To remove the effect of glucose background, a Sample blank may be set up for each reaction by omitting the Hydrolysis Enzyme Mix. The Sample blank can then be subtracted from the Sample readings.

2. Set up the Master Reaction Mix according to Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
Development Buffer	46 µL
Development Enzyme Mix	2 µL
Fluorescent Peroxidase Substrate	2 µL

3. Add 50 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.

4. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (assay blank) glycogen standard. Correct for the background by subtracting the assay bank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate glycogen standards to plot a standard curve.

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

Subtract the sample blank value from the sample readings to obtain the corrected measurement. Using the corrected measurement, the amount of glycogen present in the sample may be determined from the standard curve.

Concentration of Glycogen

$$S_a/S_v = C$$

S_a = Amount of glycogen in unknown sample (µg) from standard curve
S_v = Sample volume (µL) added into the wells
C = Concentration of glycogen in sample

Sample Calculation

Amount of glycogen (S_a) = 1.60 µg
(from standard curve)
Sample volume (S_v) = 50 µL
Concentration of glycogen in sample:
1.60 µg/50 µL = 0.032 µg/µL

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