3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Glycogen Assay Kit

Catalog Number **MAK016**
Storage Temperature –20 °C

TECHNICAL BULLETIN

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

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or 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 Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

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

Hydrolysis Assay Buffer and Development Assay 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.

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. 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 distilled 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 Assay Buffer to each well to bring the volume to 50 µL.

Glycogen Standards for Fluorometric Detection
Dilute 10 µL of the 2.0 mg/mL Glycogen Standard with 990 µL of distilled water to prepare a 0.02 mg/mL standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.02 mg/mL standard solution into a 96 well plate, generating 0 (assay blank), 0.04, 0.08, 0.12, 0.16, and 0.20 µg/well standards. Add Hydrolysis Assay Buffer to each well to bring the volume to 50 µL.

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 100 µL of water on ice. Boil homogenates for 5 minutes to inactivate enzymes. Centrifuge the samples at 13,000 × *g* for 5 minutes to remove insoluble material.

Bring samples to a final volume of 50 µL 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.

Notes: 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.

Assay Reaction
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

$$1.60\ \mu g/50\ \mu L = 0.32\ \mu g/\mu L$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

LS,MAM 09/12-1