Technical Bulletin

Glycogen Assay Kit

Catalogue number MAK604

Product Description

Glycogen is a branched polysaccharide of glucose units linked by α-1,4 glycosidic bonds and α-1,6 glycosidic bonds. It is stored primarily in the liver and muscle and forms an energy reserve that can be quickly mobilized to meet a sudden need for glucose. The most common glycogen metabolism disorder is found in diabetes, in which liver glycogen can be abnormally accumulated or depleted due to abnormal amounts of insulin. Genetic glycogen storage diseases have been associated with various inborn errors of metabolism caused by deficiencies of enzymes necessary for glycogen synthesis or breakdown.

The Glycogen Assay Kit uses a coupled enzyme reaction, which produces a colorimetric (570 nm) / fluorometric (λ_{ex}=535/λ_{em}=587nm) product, directly proportional to the glycogen concentration in the sample. The kit is suitable for the quantitative determination of glycogen in biological samples such as tissue and cells, as well as the evaluation of drug effects on glycogen metabolism.

For convenient data analysis, an assay calculator is available on the webpage for MAK604.

Components

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

- Hydrolysis Buffer — 25 mL
  Catalog number MAK604A
- Development Buffer — 25 mL
  Catalog number MAK604B
- Fluorescent Peroxidase Substrate in DMSO — 0.25 mL
  Catalogue number MAK604C
- Hydrolysis Enzyme Mix — 1 Vial
  Catalogue number MAK604D
- Development Enzyme Mix — 1 Vial
  Catalogue number MAK604E
- Glycogen Standard, 2 mg/mL — 0.12 mL
  Catalogue number MAK604F

Reagents and Equipment Required but Not Provided

- 96-well flat-bottom plate.
  - Clear plates for colorimetric assays (Catalogue number M2936 or equivalent)
  - Black plates with clear bottoms for fluorescence assays (Catalogue number CLS3631 or equivalent)
  - Cell culture or tissue culture treated plates are not recommended.
- Plate reader that is able to read absorbance and/or fluorescence.
- Pipettors and Pipettes
- Vortex Mixer
- Plate shaker (optional).

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 product is shipped on dry ice. Store at -20° C upon receipt, protected from light.

Preparation Instructions

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

Hydrolysis Buffer (MAK604A)
Allow buffer to come to room temperature before use.

Development Buffer (MAK604B)
Allow buffer to come to room temperature before use.

Fluorescent Peroxidase Substrate in DMSO (MAK604C)
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. Use within 2 months.
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 (MAK604D)
Reconstitute with 270 µL of Hydrolysis Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Development Enzyme Mix (MAK604E)
Reconstitute with 270 µL of Development Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Glycogen Standard, 2 mg/mL (MAK604F)
Thaw the solution at room temperature prior to use. Store protected from moisture at -20 °C.

Procedure

All samples and standards should be run in duplicate. Use ultrapure water for the preparation of samples.

Glycogen Standards for Colorimetric Detection

1. 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.
2. 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.
3. Add Hydrolysis Buffer to each well to bring the volume to 50 µL.

Glycogen Standards for Fluorometric Detection

1. Prepare a 0.2 mg/mL Glycogen Standard as for the colorimetric assay.
2. Dilute 10 µL of the 0.2 mg/mL Glycogen Standard solution with 90 µL of the ultrapure water to prepare a 0.02 mg/mL Glycogen Standard Solution. Detection sensitivity is 10 to 100-fold higher for a fluorometric assay.
3. 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 0.2 µg/well standards.
4. Add Hydrolysis Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation

Use ultrapure water for the preparation of samples.

Both the colorimetric and fluorometric assays require 2-50 µL of sample for each reaction (well). Liquid Samples may be assayed directly.

Endogenous compounds may interfere with the assay. To ensure accurate determination of glycogen in the test samples, it is recommended to spike samples with a known amount of Glycogen Standard (example 0.8 µg for colorimetric and 0.08 µg for fluorometric).

Tissues (10 mg) or cells (~2 X 10^6) can be homogenized in 200 µL of ice-cold water followed 10 minutes boiling at 90 °C to inactivate enzymes. Centrifuge the samples at 4 °C, 18,000 × g for 10 minutes to remove insoluble material. Collect the supernatant and dilute with Hydrolysis Buffer before assay.

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.

Add 2–50 µL of samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 50 µL with Hydrolysis Buffer.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the range of the standard curve.

A sample control (Background Sample) should be performed for each reaction by omitting the Hydrolysis Enzyme Mix. The background should be subtracted from all sample readings.

Assay Reaction

1. Add 2 µL of Hydrolysis Enzyme Mix to each sample and standard reaction (well). Do not add Hydrolysis Enzyme Mix to background sample wells. Mix well, cover the plate, and incubate for 30 minutes at room. For optimal results, shake during incubation.
2. Set up the Master Reaction Mix according to Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well). It is recommended to prepare an additional reaction mix for every six reactions. Therefore, for an experiment involving six samples, you should prepare enough mix for seven samples.

Table 1.
Master Reaction Mix

Reagent	Sample, Background, and Standard Wells
Development Buffer	46 µL
Fluorescent Peroxidase Substrate	2 µL
Development Enzyme Mix	2 µL

3. Add 50 µL of the Master Reaction Mix to each sample, glycogen standard, and background sample well and mix.
4. Incubate the plate for 30 minutes with shaking at room temperature. Protect the plate from light during the incubation.
5. For colorimetric assay, measure the absorbance at 570 nm (A570). For fluorometric assay, measure fluorescence intensity (λ_{ex}=535/λ_{em}=587 nm).

Results

Calculations

Correct Assay Values

The background for the assay is the value obtained for the 0 (blank assay) Glycogen

Standard. Blank assay values can be significant and must be subtracted from all readings (sample, sample spike, sample background, and sample standards).

Correct Samples Values

Subtract the sample background control value from the sample readings (including sample spiked if relevant).

Use the values obtained from the appropriate glycogen standards to plot a standard curve. The amount of glycogen present in the samples may be determined from the standard curve.

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

Concentration of Glycogen

DF * Sa/Sv = C

DF = Dilution factor for the sample, applied if the sample is diluted to ensure it falls within the range of the standard curve before setting up the reaction wells.

Sa = Quantity of glycogen in the unknown sample from standard curve.

Sv = Sample volume (µL) added into the wells.

C = Concentration of glycogen in sample (µg/µl).

Example Calculation

Amount of glycogen (Sa) = 0.584 µg (from standard curve).

Sample volume (Sv) = 50.0 µL.

Concentration of glycogen in sample:

0.584 µg/50.0 µL = 0.0117 µg/µL.

For samples spikes, correct for interference by using the following equation (for colorimetric and fluorometric):

$$M = \text{Glycogen Spike (µg)} * DF * \left(\frac{(OD_{Sample\ corrected})}{(OD_{Spiked\ corrected}) - (OD_{Sample\ corrected})}\right)$$

M= Amount of glycogen in sample well (µg)

Glycogen Spike (µg) = quantity of glycogen added (µg) to the sample well.

DF = Dilution factor for the sample, applied if the sample is diluted to ensure it falls within the range of the standard curve before setting up the reaction wells.

OD sample corrected = OD/λ of the sample after subtracting the blank assay and sample background readings.

OD spike corrected = OD/λ of the sample after subtracting the blank assay and sample background readings.

Example for Sample Spike Calculation

Glycogen Spike (µg) = 0.8 µg glycogen (4µl of 0.2mg/ml Glycogen standard)

DF=100

OD Blank Assay =0.062

OD Sample Background =0.277

OD Sample=0.297

OD Sample Spike=0.981

Sample volume: 50 µl

Concentration of glycogen in sample:

OD sample corrected= (0.297-0.062) -(0.277-0.062) =0.02

OD spike corrected= (0.981-0.062) -(0.277-0.062) =0.614

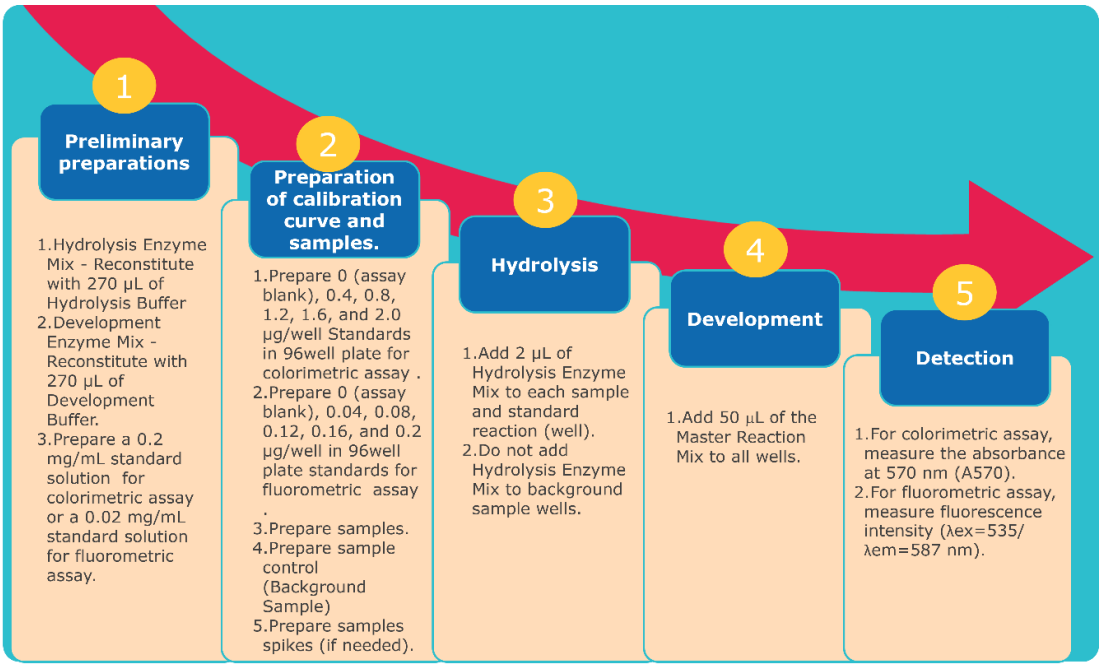
Place in the equation:

M=100*0.8 µg* 0.02/ (0.614-0.02) =2.7 µg/well

For concentration, please divide by sample volume:

C=2.7 µg /50 µl = 0.054 µg/µl

For additional assistance, please use the calculator available for the MAK604 Glycogen Assay Kit on the product webpage.



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