Technical Bulletin

Glycogen Assay Kit

Catalogue number MAK465

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. Simple, direct and automation-ready procedures for measuring glycogen concentrations find wide applications in research and drug discovery.

The Glycogen Assay Kit uses a single working reagent that combines the enzymatic break down of glycogen and the detection of glucose in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at λ_{Ex} =530 nm/λ_{Em}=585 nm is directly proportional to the glycogen concentration in the sample. This simple convenient assay is carried out at room temperature and takes only 30 minutes.

The linear detection range of the kit is 2 to 200 µg/mL glycogen for colorimetric assays and 0.2 to 20 µg/mL for fluorometric assays.

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.

Components

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

- Assay Buffer — 12 mL
  Catalogue Number MAK465A
- Dye Reagent — 120 µL
  Catalogue Number MAK465B
- Enzyme A — 1 vial
  Catalogue Number MAK465C
- Enzyme B — 120 µL
  Catalogue Number MAK465D
- Standard (50 mg/mL) — 50 µL
  Catalogue Number MAK465E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Flat bottom, clear (colorimetric assay) or black (fluorometric assay) 96-well plate for enhanced sensitivity. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes.
- Refrigerated microcentrifuge capable of RCF ≥ 14,000 × *g*
- Dounce tissue grinder set (Catalogue Number D9063 or equivalent)
- Citric acid
  (Catalogue Number 251275 or equivalent)
- Sodium fluoride (NaF)
  (Catalogue Number 201154 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. 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.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use. Keep Enzyme A and Enzyme B on ice during use.

Enzyme A: Reconstitute vial with 120 µL of Assay Buffer. Make sure Enzyme A is fully dissolved by pipetting up and down. Store reconstituted Enzyme A at -20 °C and use within 1 month after reconstitution.

Procedure

All samples and standards should be run in duplicate.

Tissue and Cell Sample Preparation

1. SH-group containing reagents (such as, DTT, β-mercaptoethanol) may interfere with this assay and should be avoided in sample preparation.
2. Briefly homogenize ~20 mg of tissue in 1 mL of 25 mM citric acid, pH 4.2, 2.5 g/L NaF on ice or 5 x 10^6 of cells in 0.5 mL of 25 mM citric acid, pH 4.2, 2.5 g/L NaF on ice.
3. Centrifuge at 14,000 × *g* for 5 minutes at room temperature to remove debris.
4. Transfer 10 µL of the clear supernatant Sample into separate wells of a clear (colorimetric) or black (fluorometric) flat-bottom 96-well plate.
5. If the Sample contains glucose, transfer an additional 10 µL of Sample to another well for use as the Sample Blank.

Colorimetric Standard Curve Preparation

1. Prepare a 200 µg/mL Glycogen Standard by mixing 5 µL of the 50 mg/mL Standard with 1.245 mL of purified water.
2. Prepare Glycogen Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Colorimetric Standards

No.	200 µg/mL Glycogen Standard	Purified Water	Glycogen (µg/mL)
1	200 µL	-	200
2	150 µL	50 µL	150
3	100 µL	100 µL	100
4	50 µL	150 µL	50
5	-	200 µL	0 (Blank)

3. Mix well and transfer 10 µL of each Standard into separate wells of the plate.

Fluorometric Standard Curve Preparation

1. Prepare a 200 µg/mL Glycogen Standard by mixing 5 µL of the 50 mg/mL Standard with 1.245 mL of purified water.
2. Prepare Glycogen Standards in 1.5mL microcentrifuge tubes according to Table2.

Table 2.
Preparation of Fluorometric Standards

No.	200 µg/mL Glycogen Standard	Purified Water	Glycogen (µg/mL)
1	20 µL	180 µL	20
2	15 µL	185 µL	15
3	10 µL	190 µL	10
4	5 µL	195 µL	5
5	-	200 µL	0 (Blank)

3. Mix well and transfer 10 µL of each Standard into separate wells of the plate.

Working Reagent

1. Mix enough reagents for the number of assays to be performed. For each Sample and Standard well, prepare 93 µL of Working Reagent according to Table 3. For each Sample Blank well, prepare 92µL of Blank Working Reagent according to Table 3.

Table 3.
Preparation of Working Reagents

Reagent	Working Reagent	Blank Working Reagent
Assay Buffer	90 µL	90 µL
Enzyme A	1 µL	-
Enzyme B	1 µL	1 µL
Dye Reagent	1 µL	1 µL

2. Transfer 90 µL of Working Reagent into each Standard and Sample well. Transfer 90 µL of Blank Working Reagent into each Sample Blank well. Tap plate to mix.

Measurement

1. Incubate the plate for 30 minutes at room temperature.
2. Read optical density (OD) at 570 nm for colorimetric assay or fluorescence intensity (F) at $\lambda_{Ex} = 530$ nm/$\lambda_{Em} = 585$ nm for fluorometric assay.

Results

1. Calculate ΔOD or ΔF by subtracting the blank reading (OD or fluorescence intensity F) of Standard #5 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD or ΔF against standard concentrations.
3. Determine the slope of the standard curve and calculate the glycogen concentration of the sample.

$$\text{Glycogen (µg/mL)} = \frac{R_{Sample} - R_{Blank}}{\text{Slope}}$$

where:

R_{Sample} = OD or fluorescence intensity (F) reading of Sample

R_{Blank} = OD or fluorescence intensity (F) reading of Blank or Sample Blank

Figure 1.
Typical Colorimetric Glycogen Standard Curve

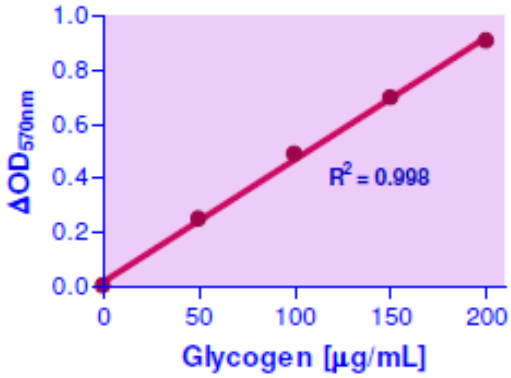
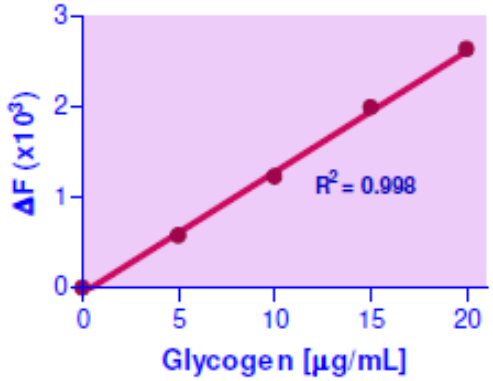


Figure 2.
Typical Fluorometric Glycogen Standard Curve



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