

Glycogen Assay Kit (Fluorometric Method)

G1373331

Storage -20°C. Protect from light.

Shipping Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Product Introduction

Glycogen is converted to glucose by amyloglucosidase, and the glucose is then oxidized by glucose oxidase to generate hydrogen peroxide. In the presence of horseradish peroxidase, Amplex Red reacts with hydrogen peroxide to produce the strongly red-fluorescent compound resorufin. The fluorescence intensity of resorufin is directly proportional to the glycogen content in the sample, and the glycogen concentration can be calculated from the fluorescence intensity of resorufin.

Components

G1373331	Component Name	Appearance	100 T	Storage	Quantity Per Test
G1373331A	Assay Buffer	Liquid	20 mL	-20°C.	As needed
G1373331B	Amyloglucosidase	Liquid	200 µL	-20°C. Store in the dark	2 µL
G1373331C	Glucose Oxidase	Liquid	200 µL	-20°C. Store in the dark	2 µL
G1373331D	Horseradish Peroxidase	Liquid	200 µL	-20°C. Store in the dark	2 µL
G1373331E	Amplex Red	Liquid	200 µL	-20°C. Store in the dark	2 µL
G1373331F	Glycogen Standard (2 mg/mL)	Liquid	600 µL	-20°C. Store in the dark	6 µL

Usage Protocol

1. Sample Preparation

- 1) Tissue samples: Add double-distilled water to the tissue sample at a ratio of tissue mass (g) to double-distilled water volume (mL) of 1:9 (e.g., 0.1 g tissue sample with 0.9 mL double-distilled water). Homogenize mechanically at 4 °C. Immediately place the obtained homogenate in a boiling water bath for 10 min, then cool to room temperature under running water. Centrifuge at 12,000 × g for 10 min at 4 °C, and collect the supernatant on ice for testing.

- 2) Cell samples: Collect 1×10^6 cells, add 200 μL double-distilled water, and homogenize mechanically at 4°C . Immediately place the obtained homogenate in a boiling water bath for 10 min, then cool to room temperature under running water. Centrifuge at $12,000 \times g$ for 10 min at 4°C , and collect the supernatant on ice for testing.
- 3) Serum (plasma) samples: Measure directly..

2. Standard Curve Setup

Take 8 EP tubes. Add 6 μL of glycogen standard (2 mg/mL) and 294 μL of assay buffer to the first tube to dilute the glycogen standard to 40 $\mu\text{g/mL}$. Add 150 μL of assay buffer to each of the remaining tubes. Transfer 150 μL from the first tube to the second tube, and continue this serial dilution through the subsequent tubes. The last tube contains 150 μL of assay buffer. The resulting glycogen concentrations in each tube are 40, 20, 10, 5, 2.5, 1.25, 0.625, and 0 $\mu\text{g/mL}$, respectively.

3. Standard and Sample Assay

- 1) Add 50 μL of the serially diluted standards and 50 μL of samples to a black 96-well plate.
- 2) Prepare the Reaction Working Solution according to the number of standard wells and sample wells. Each well requires 50 μL of Reaction Working Solution, consisting of 2 μL of amyloglucosidase, 2 μL of glucose oxidase, and 46 μL of assay buffer. After preparation, add 50 μL per well and incubate at 37°C for 30 min.
- 3) Prepare the Chromogenic Working Solution according to the number of standard wells and sample wells. Each well requires 50 μL of Chromogenic Working Solution, consisting of 2 μL of horseradish peroxidase, 2 μL of Amplex Red, and 46 μL of assay buffer. After preparation, add 50 μL per well and incubate at 37°C for 30 min.
- 4) Measure fluorescence intensity with excitation at 535 nm and emission at 587 nm.
- 5) Note: The presence of H_2O_2 may interfere with glycogen detection. If the sample contains H_2O_2 , set up a background control well in parallel, and add the Reaction Working Solution without glucose oxidase (replace the 2 μL of glucose oxidase with assay buffer when preparing the Reaction Working Solution).

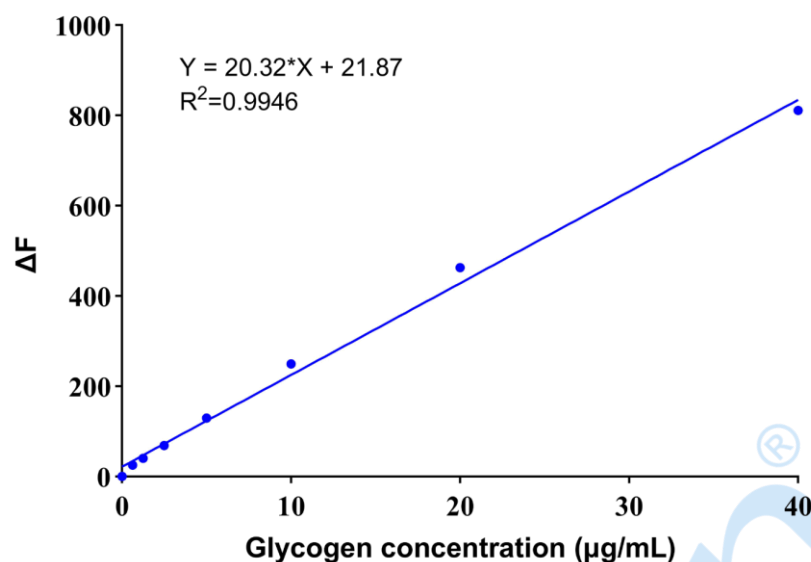
4. Result Calculation

The fluorescence intensity of the blank well, standard wells, and sample wells are recorded as F_{blank} , F_{standard} , and F_{sample} , respectively. $\Delta F_{\text{standard}} = F_{\text{standard}} - F_{\text{blank}}$; $\Delta F_{\text{sample}} = F_{\text{sample}} - F_{\text{blank}}$.

If the background signal of the sample is relatively high, subtract the signal value of the sample background control from that of the sample.

Plot a standard curve with the glycogen standard concentration as the abscissa and the fluorescence signal intensity ($\Delta F_{\text{standard}}$) as the ordinate. Substitute ΔF_{sample} into the standard curve to calculate the glycogen concentration in the sample.

5. Results Presentation



Precautions

1. Thaw the Enzyme Working Solution on ice. Avoid leaving it at room temperature before and after use.
2. Before formal testing, it is recommended to perform a preliminary experiment using 2–3 samples with expected large differences.
3. Amplex Red is relatively unstable in the air. Use it as soon as possible after opening, and take care to protect it from light during use.
4. For fluorescence detection, a black 96-well plate is recommended.
5. Biochemical assay reagents generally have irritancy and biological toxicity. For your safety and health, please take proper biological safety precautions throughout the experiment.