# Glycogen

# Fluorometric Microplate Assay Kit

# User Manual

## Catalog # CAK8015

(Version 1.2A)

Detection and Quantification ofGlycogen ContentinTissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

**For research use only. Not for diagnostic or therapeutic procedures.**

## I. INTRODUCTION

Glycogen is a multibranched polysaccharide of glucose that serves as a form of energy storage in humans, animals, and fungi. The polysaccharide structure represents the main storage form of glucose in the body.

In humans, glycogen is made and stored primarily in the cells of the liver and the muscles, hydrated with three or four parts of water. Glycogen functions as the secondary long-term energy storage, with the primary energy stores being fats held in adipose tissue. Muscle glycogen is converted into glucose by muscle cells, and liver glycogen converts to glucose for use throughout the body including the central nervous system.

Glycogen Fluorometric Microplate Assay Kit is a sensitive assay for determining Glycogen in various samples. In the assay, glucoamylase hydrolyzes the glycogen to glucose which is then specifically oxidized to produce a compound that reacts with the probe, which can be detected fluorometrically (Ex/Em 535/587).

## II.KIT COMPONENTS

| Component | Volume | Storage |
|---|---|---|
| 96-Well Microplate | 1 plate | -- |
| Assay Buffer | 30 ml x 4 | 4 °C |
| Reaction Buffer | 20 ml x 1 | 4 °C |
| Enzyme | Powder x 1 | -20 °C, keep in dark |
| Probe | Powder x 1 | -20 °C, keep in dark |
| Probe Diluent | 1 mlx 1 | 4 °C |
| Standard | Powder x 1 | 4 °C |
| Plate Adhesive Strips | 3 Strips | -- |
| Technical Manual | 1 Manual | |

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microplate reader to read fluorescence at Ex/Em = 535/587
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

## IV. REAGENT PREPARATION

**Standard**:Briefly centrifuge prior to opening.Dissolvein1 mlAssay Bufferto generate2mg/ml of standardstock solution,store at 4°C for 2 days or -20°C for 4-5 monthsafter reconstitution.Then dilute to0.1mg/mlstandard top solutionby adding 50 µl stock solution into 950µl distilled water.Perform 2-fold serial dilutions of the top standardsolution usingAssay Bufferto make the standard curve.The concentration of standard curve could be100/50/25/12.5/6.25/3.12/1.56µg/ml.

**Enzyme:**Add 5 ml Reaction Buffer to dissolve before use.Aliquot & store at -20 °C.Use within 1 month.

**Probe:** Warm Probe Diluent to RT prior to use to melt frozen Probe Diluent; then add 1 ml Probe Diluent to dissolve. Store at -20 °C, protect from light and moisture. Use within 1 month.

**Note:** Divide into small aliquots to avoid repeated freeze-thaw cycles.

## V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer for 5 ×$10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); boil the lysates for 10 minutes, centrifuged at 10,000g 4°C for 10minutes, transfer the supernatant into a new centrifuge tube for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer;boil thehomogenates for 10 minutes;centrifuged at 10,000g for 10 minutes, transfer the supernatant into a new centrifuge tube for detection.

3.For liquid samples

Detect directly or dilute with Assay Buffer.

## VI. ASSAY PROCEDURE

Warm thesolution to room temperature before use.

Add following reagents in the microplate:

| Reagent* | Sample** | Standard | Blank |
|---|---|---|---|
| Reaction Buffer | 170 µl | 170 µl | 170 µl |
| Sample | 10 µl | -- | -- |
| Standard | -- | 10 µl | -- |
| Distilled water | -- | -- | 10 µl |
| Probe | 10 µl | 10 µl | 10 µl |
| Enzyme | 10 µl | 10 µl | 10 µl |
| Mix, put it in the oven, 37 °C for 10 minutes,protected from light, record fluorescence measured at Ex/Em = 535/587 nm. | | | |

**Note:**

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

## VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(RFU_{Sample}-RFU_{Blank})-Intercept}{Slope}\times n(\mu g/ml)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard RFU and concentration

2.1According to the quantity of cells or bacteria

$$C=\frac{(C_{Standard}\times V_{Standard})\times(RFU_{Sample}-RFU_{Blank})}{(RFU_{Standard}-RFU_{Blank})\times N\times(V_{Sample}/V_{Assay})}(\mu g/10^4)$$

2.2 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(RFU_{Sample}-RFU_{Blank})}{(RFU_{Standard}-RFU_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu g/g)$$

2.3According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(RFU_{Sample}-RFU_{Blank})}{(RFU_{Standard}-RFU_{Blank})\times V_{Sample}}(\mu g/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, μg/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

$V_{Sample}$: the volume of sample in assay procedure, μl

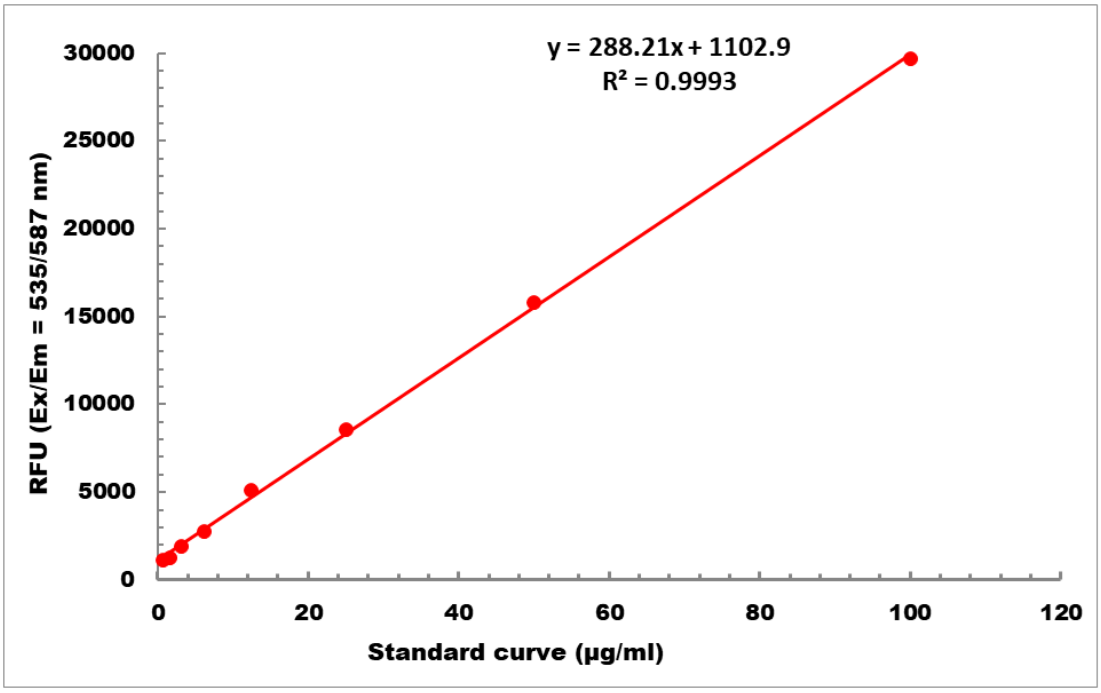
$V_{Assay}$: the volume of Assay Buffer, μl

W: the weight of sample, g

N: the quantity of cell or bacteria, $10^4$

## VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.




Detection Range: 1 µg/ml - 100 µg/ml