Glycogen Colorimetric Assay Kit (384-well) (#BN01120)

(Catalog # BN01120; 400 assays; Store at -20°C)

I. Introduction:

Glycogen is a branched polysaccharide, made of glucose units that serves as the primary source of energy storage in animals. Most of the glucose residues in glycogen are linked by α-1,4-glycosidic bonds with branching via α-1,6 linkage. In mammals, the two major sites of glycogen storage are liver and skeletal muscle. In liver, glycogen synthesis and degradation are regulated to maintain blood-glucose levels, while stored glycogen in muscle can be processed to meet the energy needs of the muscle itself. Abnormal ability to utilize glycogen is found in patients suffering diabetes and other genetic glycogen storage related diseases. Assay Genie's Glycogen Colorimetric Assay Kit is an easy and convenient assay to measure glycogen levels in biological samples. In the assay, glucoamylase hydrolyzes the glycogen releasing glucose which is then specifically oxidized to produce a product that reacts with GenieRed Probe to generate color (OD 590 nm). The method is quantitative, rapid, simple, sensitive, and designed for high throughput format. The assay can detect as low as 0.025 µg of glycogen in 384-well assay format.

II. Application:

- Measurement of Glycogen in various tissues/cells
- Analysis of metabolism and cell signaling in various cells

III. Sample Type:

- Animal tissues such as liver etc.
- Cell culture: adherent or suspension cells

IV. Kit Contents:

Components	BN01120	Cap Code	Part Number
Hydrolysis Buffer	25 ml	NM	BN01120-1
Development Buffer	25 ml	WM	BN01120-2
GenieRed Probe	0.8 ml	Red	BN01120-3
Hydrolysis Enzyme Mix	1 vial	Blue	BN01120-4
Development Enzyme Mix	1 vial	Green	BN01120-5
Glycogen Standard (2.0 mg/ml)	100 µl	Yellow	BN01120-6

V. User Supplied Reagents and Equipment:

- 384-well clear plate with flat bottom
- Multi-well spectrophotometer with 384-well plate reading capability

VI. Storage and Handling:

Store kit at –20°C, protected from light and moisture. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **Hydrolysis Buffer and Development Buffer:** Warm to room temperature before use. Store at –20°C or 4°C.
- **GenieRed Probe:** Ready to use as supplied. Warm to room temperature to melt the GenieRed in DMSO before use. Mix well, store at –20°C. Protect from light and moisture. Aliquot to avoid multiple freeze thaws. Use within 2 months.
- **Hydrolysis Enzyme Mix:** Reconstitute with 880 µl of Hydrolysis Buffer. Vortex gently to dissolve. Keep on ice. Store at –20°C. Use within two months.
- **Development Enzyme Mix**: Reconstitute with 880 µl of Development Buffer. Vortex gently to dissolve. Keep on ice. Store at –20°C. Use within two months

VIII. Glycogen Assay Protocol:

1. **Sample Preparation:** Liquid samples can be assayed directly. For cells, homogenize 10^6 cells or 5 mg tissue with 200 µl dH_2O on ice. Boil the homogenates for 10 min to inactivate the endogenous enzymes present in the sample. Spin the boiled samples at 18,000 x g for 10 min to remove the insoluble material. The supernatant is ready to be assayed. Add 1-5 µl samples to a 384-well clear plate. Adjust the volume to 12 µl/well with Hydrolysis Buffer.

   Notes:

   a. For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions to ensure the readings are within the Standard Curve range.

   b. Glycogen can be metabolized very rapidly in some tissues after death (within a min); therefore special care must be taken to minimize glycogen loss when preparing tissue samples. Freezing samples or keeping them on ice can minimize glycogen loss due to endogenous metabolism.

   c. For samples having glucose background, prepare parallel well(s) as background controls containing same amount of sample as in the test well. (See section 3).

   d. Endogenous compounds may interfere with the reaction. To ensure accurate determination of Glycogen in the test samples, we recommend spiking samples with a known amount of Standard (0.2 µg).

   e. There are various methods for extracting glycogen from tissues. We strongly recommend consulting the literature to determine the best method for your purposes. However, for convenience, a few methods taken from literature are described on page 3.

   f. Instrument reader settings need to be adjusted according to the chosen 384-well clear plate. (The right dimension of the 384-well plate in use may be available in the manual provided by the plate-manufacturer).

2. **Standard Curve Preparation:** Dilute the Glycogen Standard to 0.05 mg/ml by adding 5 µl of the Standard to 195 µl of distilled water, mix well. Add 0, 2, 4, 6, 8, 10 µl to a series of wells. Adjust volume to 12 µl/well with Hydrolysis Buffer to generate 0, 0.1, 0.2, 0.3, 0.4 and 0.5 µg per well of the Glycogen Standard.
3. **Hydrolysis:** Add 2 µl Hydrolysis Enzyme mix to each Standards and samples, mix well. Incubate for 1 hour at 37°C.

   Note: Endogenous glucose generates background readings. If glucose is present in your sample, you may need to run a glucose background control in a separate well by adding 2 µl Hydrolysis Assay Buffer instead of Hydrolysis Enzyme mix in order to determine the level of glucose background in your sample. The glucose background then can be subtracted from glycogen readings.
4. **Reaction Mix:** Mix enough reagents for the number of samples and Standards to be performed: For each well, prepare a total of 16 µl Reaction Mix containing:

Development Buffer	12 µl
Development Enzyme Mix	2 µl
GenieRed Probe	2 µl

   Add 16 µl of the Reaction Mix to each well containing the Glycogen Standard or samples, mix well.
5. **Measurement:** Incubate the reaction for 1 hour at 37°C, protected from light. Measure absorbance (OD: 590 nm).
6. **Calculation:** Correct background by subtracting the 0 Glycogen Standard from all Standard readings. Plot Glycogen Standard Curve. Apply sample readings to the standard curve to get B µg of glycogen in the sample wells. (**Note:** If the sample glucose background reading is significant then must be subtracted from all the sample readings).

Sample Glycogen concentration (C) = B/V X D µg/µl

Where: **B** is the amount of glycogen from Standard Curve (µg)
V is the sample volume added into the reaction well (µl)
D is the sample dilution factor

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, Glycogen amount in sample well (B) = $\left(\frac{(OD_{sample\ (corrected)})}{(OD_{sample\ +\ glycogen\ Std(corrected)})-(OD_{sample(corrected)})}\right)$ * Glycogen Spike (µg)

Glycogen molecular size: ~ 60,000 glucose molecules (MW ~10^6-10^7daltons).
Glucose Molecular Weight: 180.16.

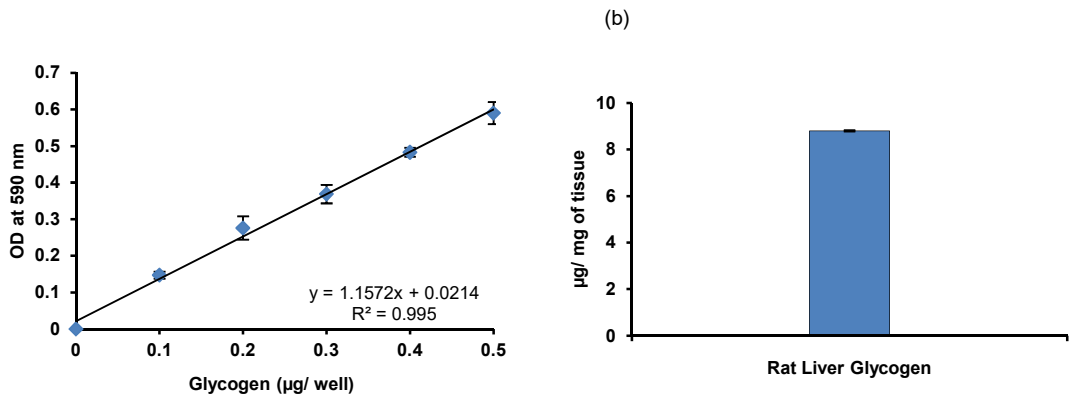

Figure: (a) Glycogen Standard Curve: **(b)** Glycogen in rat liver lysate. Rat liver (17 mg) was homogenized with 680 µl of deionized water, boiled for 10 min, and then centrifuged for 10 min. at 18000 x g. Supernatant was collected. Supernatant was diluted 10 times and 5 µl was used for the assay following the kit's protocol. Sample was spiked with 0.2 µg of Glycogen Standard.

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