EZScreen™ Glycogen Colorimetric Assay Kit (384-well)

rev. 12/17

(Catalog # K960-400; 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. BioVision EZScreen™ 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 OxiRed 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	K960-400	Cap Code	Part Number
Hydrolysis Buffer	25 ml	NM	K960-400-1
Development Buffer	25 ml	WM	K960-400-2
OxiRed Probe	0.8 ml	Red	K960-400-3
Hydrolysis Enzyme Mix	1 vial	Blue	K960-400-4
Development Enzyme Mix	1 vial	Green	K960-400-5
Glycogen Standard (2.0 mg/ml)	100 µl	Yellow	K960-400-6

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.
- **OxiRed Probe:** Ready to use as supplied. Warm to room temperature to melt the OxiRed 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
OxiRed 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

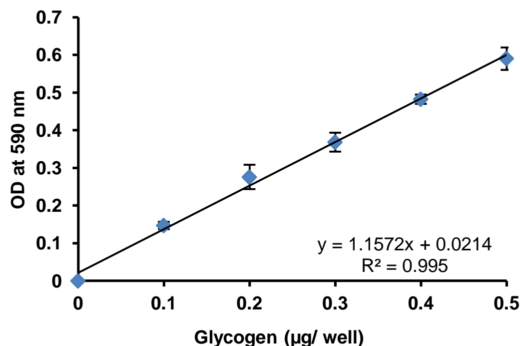
Where: **B** is the amount of glycogen from Standard Curve (µg)
V is the sample volume added into the reaction well (µl)
D is the sample dilution factor

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) $= \left(\frac{(OD_{\text{sample (corrected)}})}{(OD_{\text{sample + glycogen Std(corrected)}})-(OD_{\text{sample(corrected)}})}\right)$ * Glycogen Spike (µg)

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

(a)



(b)

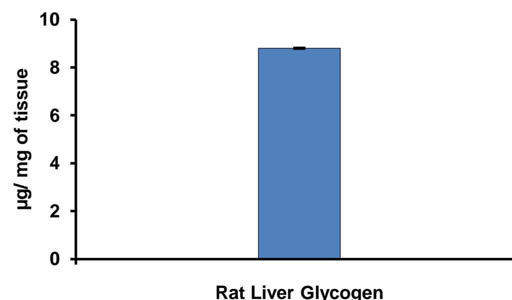


Figure: (a) Glycogen Standard Curve: **(b)** Glycogen in rat liver lysate. Rat liver (17 mg) was homogenized with 680 µl of deionized water, boiled for 10 min, and then centrifuged for 10 min. at 18000 x g. Supernatant was collected. Supernatant was diluted 10 times and 5 µl was used for the assay following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

IX. Related Products:

Glucose Colorimetric/Fluorometric Assay Kit (K606)
PicoProbe™ Glucose Fluorometric Assay Kit (K688)
Glucose-6-Phosphate Colorimetric Assay Kit (K657)
Glucose Uptake Fluorometric Assay Kit (K666)
Glucose and Sucrose Colorimetric/Fluorometric Assay Kit (K616)
Glucose Dehydrogenase Activity Colorimetric Assay Kit (K786)
EZScreen™Glucose Colorimetric Assay kit (384 well) (K950)
Glucose Colorimetric Assay Kit II (K686)
Glucose-1-Phosphate Colorimetric Assay Kit (K697)
Phosphoglucomutase Colorimetric Assay Kit (K774)
Glucose Uptake Colorimetric Assay Kit (K676)
Maltose and Glucose Colorimetric/Fluorometric Assay Kit (K618)
Glucose Oxidase Activity Assay Kit (K788)
EZScreen™Lactate Colorimetric Assay kit (384 well) (K951)

FOR RESEARCH USE ONLY! Not to be used on humans.

Sample Preparation:

There are a variety of methods for extraction of glycogen from tissues depending upon a) the type of tissue the glycogen is to be extracted form and b) the type of information desired. The gentlest procedure is the method referred in reference 1, which maintains the molecular weight of the glycogen so that analysis of the molecular distribution is possible.

A rapid method useful for small tissue samples is detailed in reference 4. Basically a small sample of tissue is homogenized in 50 volumes of distilled water, diluted appropriately and immediately used in the assay. Since endogenous glucose will be a significant factor utilizing this method, a glucose background control must be conducted where the sample is directly placed in development buffer with development enzyme mix (without prior treatment with the hydrolysis reagents). If the sample will not be immediately assayed, it should be placed in a capped, vented microcentrifuge tube, boiled for 5 min to inactivate any enzyme activities present and stored at -20°C until assayed. Samples from high content tissues (liver, muscle) prepared in this way should have sufficient glycogen such that 1-5 µl aliquots will give a clearly measureable colorimetric signal.

Caveats:

1) In some tissues such as neural tissue, very rapid rates of anaerobic metabolism continue after death causing rapid declines in glucose to undetectable levels within a few seconds. Utilization of glycogen follows and large decreases in glycogen content are seen within less than a minute. Thus accurate measurement of glycogen in such tissues requires very rapid quenching of metabolic activity such as freeze clamp or immediate removal of tissue to liquid nitrogen followed by grinding in the liquid nitrogen and storage at -20 or -80°C until used.

2) In some samples i.e., Saccharomyces, the glycogen is distributed between soluble and insoluble pools. It is not clear that both pools are completely hydrolyzed

If the sample to be analyzed is sufficiently large (a few hundred milligrams to grams of tissue), a more quantitative method is as follows:

Take tissue or cells to a final content of 30-50% in 30% KOH. Heat to 100°C for 2 hours, cool and add 2 volumes of 95% ethanol. This will precipitate the crude glycogen. Centrifuge and collect the precipitate. Dissolve/suspend the precipitate in a minimal amount of distilled water and acidify to pH 3 with HCL (5N). Re-precipitate with 1 volume of ethanol. Repeat wash/acidification/precipitation 2 more times, then wash precipitate with ethanol and dry. This procedure removes the vast majority of the glucose background with minimal effect on the glycogen. The dried material can be weighed and dissolved/suspended in hydrolysis buffer for analysis.

X. References:

1) E.Bueding and S.A. Orrell (1964) A Mild Procedure for the Isolation of Polydisperse Glycogen from Animal Tissues. J. Biol.Chem. 239, 12, pp 4018-4020

2) R. H. Dalrymple, R. Hamm (1973) A method for the extraction of glycogen and metabolites from a single muscle sample. Intl J of Food Sci & Tech, 8, 4 pp 439-444

3) G. Cappeln, F. Jessen (2002) ATP, IMP, and Glycogen in Cod Muscle at Onset and During Development of Rigor Mortis Depend on the Sampling Location. J. Food Sci. 67, #3, pp 991-995

4) Huijing, F. (1970) A Rapid Enzymic Method For Glycogen Estimation In Very Small Tissue Samples., Clin. Chim. Acta. 30, pp 567-572.

5) Monique Rousset, etc. (1981) Presence of Glycogen and Growth related Variations in 58 Cultured Human Tumor Cell Lines. Cancer Research. 41, 1165-1170.