ab65620 – Glycogen Assay Kit

For the rapid, sensitive and accurate measurement of glycogen in various samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab65620
(use www.abcam.cn/ab65620 for China, or www.abcam.co.jp/ab65620 for Japan)

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer II/Glycogen Development Buffer	25 mL	-20°C
Assay Buffer VIII/Glycogen Hydrolysis Buffer	25 mL	-20°C
Development Enzyme Mix II/Glycogen Development Enzyme Mix (Lyophilized)	1 vial	-20°C
Hydrolysis Enzyme Mix I/Glycogen Hydrolysis Enzyme Mix (Lyophilized)	1 vial	-20°C
Glycogen Standard (2 mg/mL)	100 µL	-20°C
OxiRed Probe	200 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at OD 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)
- MilliQ water or other type of double distilled water (ddH2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom for colorimetric assay / 96 well plate with flat bottom, preferably black for fluorometric assay
- Dounce homogenizer (if using tissue)
- Protein quantification method such as BCA protein assay kit reducing agent compatible (microplate) (ab207003) or BCA protein assay kit for low concentrations (ab207002)

Optional – for tissues with large volume of sample (>100 mg):

- Potassium hydroxide (KOH), 30%
- Ethanol, 95%
- Hydrochloric acid (HCl), 5N

Reagent Preparation

- Briefly centrifuge small vials at low speed prior to opening.
- Aliquot reagents to have enough volume to perform the desired number of assays.

Assay Buffer II/Glycogen Development Buffer and **Assay Buffer VIII/Glycogen Hydrolysis Buffer:** Ready to use as supplied. Equilibrate to room temperature before use.

Development Enzyme Mix II/Glycogen Development Enzyme Mix (lyophilized): Add 220 µL Assay Buffer II/Glycogen Development Buffer to reconstitute. Vortex tubes to dissolve. Keep on ice during assay. Use within 2 months.

Hydrolysis Enzyme Mix I/Glycogen Hydrolysis Enzyme Mix (lyophilized): Reconstitute in 220 µL Assay Buffer VIII/Glycogen Hydrolysis Buffer. Gently vortex tubes to dissolve. Keep on ice during the assay. Use within two months.

Glycogen Standard (2 mg/mL): Ready to use as supplied. Keep on ice while in use.

OxiRed Probe: Warm by placing in a 37°C bath for 1–5 min to thaw the DMSO solution before use. Once thawed, use within two months. Store at -20°C protected from light.

Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

For Colorimetric Assay: Prepare 100 µL of 0.2 mg/mL Glycogen standard by diluting 10 µL of the provided standard (2 mg/mL solution) with 90 µL of ddH2O.

For Fluorometric Assay: Prepare 1 mL of 0.02 mg/mL Glycogen standard by diluting 10 µL of the provided standard (2 mg/mL solution) with 990 µL of ddH2O.

Prepare standard curve dilution in a microplate or microcentrifuge tubes as shown in the table. Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL):

Standard #	Glycogen Standard (µL)	Assay Buffer VIII/Glycogen Hydrolysis Buffer (µL)	Final volume standard in well (µL)	End amount Glycogen (µg/well)	
				Colorimetric	Fluorometric
1	0	150	50	0	0
2	6	144	50	0.4	0.04
3	12	138	50	0.8	0.08
4	18	132	50	1.2	0.12
5	24	126	50	1.6	0.16
6	30	120	50	2	0.2

Sample Preparation

- Perform several dilutions of sample to ensure readings are within standard value range.
- Use fresh samples or snap freeze samples in liquid nitrogen upon extraction and store immediately at -80°C. When you are ready to test samples, thaw on ice. Note: this might affect the stability of samples, and readings can be lower than expected.
- In some tissues anaerobic metabolism continues to happen after death, causing rapid decline in glucose levels. To accurately measure glycogen is such tissues, metabolic activity should be quenched by freeze clamping or immediate submersion of tissue in liquid nitrogen, followed by grinding in liquid nitrogen and storage at -80°C.
- Sample concentration may be determined using a standard method such as BCA.

1. **Cell (adherent or suspension)** and **Tissue (< 100 mg)** samples:
   1.1. Harvest amount of sample necessary for each assay
   Cell samples: recommendation = 1 x 10^6 cells.
   Tissue samples (< 100 mg): recommendation = 10 mg.

1.2. Wash with cold PBS.
1.3. Resuspend in 200 µL of ddH2O on ice.
1.4. **Cell samples:** Homogenize quickly by pipetting up and down a few times.
Tissue samples: Homogenize with a Dounce homogenizer or pestle sitting on ice, with 10–15 passes
1.5. Boil the homogenates for 10 minutes to inactivate enzymes in the sample. If sample will not be assayed immediately, store at -20°C. Do not store for more than 1 month.
1.6. Centrifuge boiled samples 10 minutes at 4°C at 18,000 x g in a cold microcentrifuge to remove any insoluble material.
1.7. Collect supernatant and transfer to a new tube.

2. **Tissue sample (> 100 mg)**:
2.1. Harvest the amount of tissue necessary (from few hundred milligrams to grams).
2.2. Wash tissue in cold PBS.
2.3. Resuspend tissue in 30% KOH to a final content of 30% - 50%.
2.4. Heat sample to 100°C for 2 hours.
2.5. Cool sample and add 2 volumes of 95% Ethanol to precipitate the crude glycogen.
2.6. Centrifuge for 10 minutes at 4°C at 18,000 x g in a cold microcentrifuge to remove any insoluble material. Discard supernatant.
2.7. Dissolve or resuspend the precipitate in a minimal amount of ddH2O (enough to dissolve pellet) and acidify sample to pH 3 with HCl 5N, drop by drop.
2.8. Re-precipitate with 1 volume of 95% Ethanol.
2.9. Repeat steps 2.4 – 2.8 two more times.
2.10. Wash precipitate in 95% Ethanol and dry. The dried material can be weighed and dissolved in Assay Buffer VIII/Glycogen Hydrolysis Buffer for analysis.

3. **Plasma, Serum and Urine samples**:
Add sample to the microplate assay wells. Use heparin when collecting plasma. Endogenous compounds in the sample may interfere with the reaction. To ensure accurate determination of Glycogen in the test samples, spike samples with a known amount of Standard (0.8 µg).

Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate
- Samples from tissues with high glycogen content (liver, muscle) only require 5 – 25 µL sample to give a measurable colorimetric signal. Samples from tissues with low glycogen content generally require 50 µL sample in the colorimetric assay or should be tested in the more sensitive fluorometric assay (10 – 25 µL). Glucose present in cell or tissue extracts can generate background in this assay. If you suspect your samples contain glucose, set up Sample Background Controls.

4. Set up Reaction wells:
- Standard wells = 50 µL standard dilutions.
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer VIII/Hydrolysis Buffer).
- Sample Background Control wells = 2 – 50 µL samples (adjust volume to 50µL/well with Assay Buffer VIII/Hydrolysis Buffer).

5. Assay procedure:
5.1. **For Colorimetirc Assay:** Add 2 µL of Hydrolysis Enzyme Mix I/Hydrolysis Enzyme Mix to standard and sample wells. Do not add Hydrolysis mix to background controls.
For Fluorometric Assay: Add 1 µL of Hydrolysis Enzyme Mix I/Hydrolysis Enzyme Mix to standard and sample wells. Do not add Hydrolysis mix to background controls.
5.2. Mix well and incubate at room temperature for 30 minutes.
5.3. Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction mix to ensure consistency.

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)
Assay Buffer II/Development Buffer	46	48.7
Development Enzyme Mix II/Development Enzyme Mix	2	1
OxiRed Probe	2	0.3

5.4. Add 50 µL of Reaction Mix into each standard, sample and sample background wells.
5.5. Mix and incubate at room temperature for 30 min protected from light.
5.6. Measure output immediately after incubation on a microplate reader at:
Colorimetric Assay: OD 570 nm
Fluorometric Assay: Ex/Em = 535/587 nm.

6. Calculations
6.1. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
6.2. Average the duplicate reading for each standard and sample.
6.3. Subtract the sample background control from sample reading.
6.4. Plot the corrected absorbance values for each standard as a function of the final concentration of Glycogen.
6.5. Draw the best smooth curve through these points to construct the standard curve. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
6.6. Concentration of Glycogen (µg/µL) in the test samples is calculated as:

$$\text{Glycogen concentration} = \left(\frac{Ts}{Sv}\right) * D$$

Ts = amount of glycogen in the sample well calculated from standard curve (µg).
Sv = sample volume added in the sample wells (µL).
D = sample dilution factor.

Glycogen molecular size = ~60,000 glucose molecules (10^6 – 10^7 daltons)
Glycogen molecular weight = 180.16 g/mol
6.7. For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.
6.8. For spiked samples, the concentration of Glycogen in sample well is calculated as:

$$\text{Glycogen} = \left(\frac{(\text{ODs cor})}{(\text{ODs} + \text{Ts cor}) - (\text{ODs cor})}\right) * \text{Glycogen spike (µg)}$$

ODs cor = OD sample corrected
ODs = OD sample
Ts cor = amount of glycogen from standard curve corrected

Interferences:
Reducing substances will interfere with this assay. If your sample is likely to contain reducing substances, we recommend using Glycogen Assay Kit II (ab169558).

Urine diphosphate glucose (UDP) may interfere depending on the amount of UDP glucose compared to glycogen in the sample. A background control can be run to measure any interference from endogenous glucose/UDP glucose. Oxidation of glucose in the assay leads eventually to color development. It is possible that UDP glucose competes in this late step but it has not been tested.

Technical Support

For all technical or commercial enquiries please go to:

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