ab169558 - Glycogen Assay Kit II (Colorimetric)

For the preparation of nuclear extracts from mammalian cells and tissue samples

This product is for research use only and is not intended for diagnostic use

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 the kit at -20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm assay buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

Materials Supplied:

Item	Quantity	Storage Conditions
Assay Buffer VIII/Glycogen Hydrolysis Buffer	25 mL	-20°C
Assay Buffer II/Glycogen Development Buffer	25 mL	-20°C
Hydrolysis Enzyme Mix I/Hydrolysis Enzyme Mix (Lyophilized)	1 vial	-20°C
Developer Solution III/Probe (Lyophilized)	1 vial	-20°C
Glycogen Standard/Glycogen Standard (2 mg/ml)	100 µL	-20°C
Development Enzyme Mix V/Development Enzyme Mix (Lyophilized)	1 vial	-20°C

Materials Required, Not Supplied

- 96-well clear plate with flat bottoms
- Multi-well spectrophotometer (ELISA reader)

Reagent Preparation

Hydrolysis Enzyme Mix I/Hydrolysis Enzyme Mix: Reconstitute with 220 µl Assay Buffer VIII/Glycogen Hydrolysis Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.

Development Enzyme Mix V/Development Enzyme Mix: Reconstitute with 220 µl ddH2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

Developer Solution III/Probe: Reconstitute with 220 µl dH2O. Pipette up and down to dissolve completely. Stable for 2 months at -20°C.

Glycogen Assay Protocol

Glycogen Standard Curve: Dilute Glycogen Standard to 0.2 mg/ml (0.2 µg/µl) by adding 10 µl of 2 mg/ml Glycogen Standard to 90 µl dH2O, mix well. Add 0, 2, 4, 6, 8 and 10 µl of 0.2 mg/ml Glycogen Standard into series of wells in 96 well plate to generate 0, 0.4, 0.8, 1.2, 1.6 and 2 µg/well Glycogen Standard. Adjust volume to 50 µl per well with Assay Buffer VIII/Glycogen Hydrolysis Buffer.

Sample preparation: Tissue (10 mg) or cells (1 x 10^6) should be rapidly homogenized with 200 µl ddH_2O for 10 minutes on ice. Boil the homogenates for 10 minutes to inactivate enzymes. Centrifuge at 18000 rpm for 10 minutes and remove insoluble material. Collect the supernatant. Supernatant is ready to be assayed. Add 1-50 µl samples (~50 µg) into a 96 well plate and bring the volume to 50 µl with Assay Buffer VIII/Glycogen Hydrolysis Buffer.

***Note**: For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the Standard Curve range.*

Glucose in samples will generate background. If your sample has significant amount of glucose, a sample background control is required.

Hydrolysis: Add 2 µl of Hydrolysis Enzyme Mix I/Hydrolysis Enzyme Mix to Standard and samples, mix well. Incubate at room temperature for 30 minutes.

***Note**: Don't add Hydrolysis Enzyme Mix I/Hydrolysis Enzyme Mix to the sample background control.*

Reaction Mix: Mix enough reagents for the number of samples and standards to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
Assay Buffer II/Glycogen Development Buffer	44 µl	46 µl
Development Enzyme Mix V/Development Enzyme Mix	2 µl	2 µl
Developer Solution III/Probe	2 µl	2 µl

Add 48 µl of the Reaction Mix to each well containing the Standard and samples and 50 µl of Background Control Mix to background control well.

Measurement: Incubate at room temperature for 30 minutes. Measure OD450 nm with a microplate reader.

Data Analysis

Calculation: Subtract 0 Glycogen Standard reading from all readings. Plot the Glycogen Standard curve. If background control reading is significant, subtract the background control reading from sample reading. Apply the corrected sample reading to the Glycogen Standard curve to get B µg of Glycogen in the samples.

$$\text{Sample glycogen concentration (C)} = \frac{B}{V} \times \text{Dilution Factor} = \mu g/\mu l = mgml$$

Where:

B is the Glycogen amount from Standard Curve (µg)

V is the sample volume used in the reaction well (µl).

Sample glycogen concentration can also be expressed in µg/mg of sample or other desired method.

Glycogen molecular weight ~ 10^5-10^7 g/mo

Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)