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ab235633 O-GlcNAc Modified Glycoprotein Assay Kit (FACS/Microscopy, Green Fluorescence)

For the measurement of O-GlcNAc-glycosylated proteins in suspension or adherent cell cultures.

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.

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1. Overview

The O-GlcNAc Modified Glycoprotein Assay Kit (FAC/Microscopy, Green Fluorescence) (ab235633) is a highly specific, simple and robust method for labeling and detection of O-GlcNAc-glycosylated proteins within cells. The kit uses a modified glucosamine precursor that is fed directly into the cells, processed by the hexosamine pathway and incorporated into the protein. Followed by click reaction with alkyne-containing dye, this system offers a powerful method for imaging the localization, trafficking, and dynamics of glycans, or detection by FACS for quantitative studies. Labeled Glycoproteins can be directly detected in 1D or 2D gels using the appropriate excitation sources, or enriched by immunoprecipitation with biotin-alkyne or antibodies prior to proteomic analysis.

Seed the cell suspension directly into tissue culture vessels.

Next day, add1X GlcAz Label. Add treatments and incubate for 1-3 days in a 37 °C incubator.

Remove the media and pellet the suspension cells at 300 x *g* for 5 minutes. Gently remove the media.

Add 100 µL of Fixative Solution and incubate cells for 15 minutes at RT protected from light.

Wash the cells and incubate with 100 µL of 1X Permeabilization Buffer for10 minutes at RT.

Incubate with 100 µL of 1X Reaction for 30 minutes at RT protected from light. Wash and proceed to DNA staining if desired.

Analyze cells by fluorescence microscopy or FACS

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
GCK Assay Buffer/Wash Buffer (10X)	25 mL	-20°C	4°C
Fixative Solution I/Fixative Solution	10 mL	-20°C	4°C
10X Permeabilization Buffer/Permeabilization Buffer (10X)	25 mL	-20°C	4°C
1000X GlcAz Label/GlcAz Label (1000X)	10 µL	-20°C	-20°C
100X Copper Reagent/Copper Reagent (100X)	100 µL	-20°C	-20°C
100X Fluorescent Alkyne I/Fluorescent Alkyne (100X)	100 µL	-20°C	-20°C
20X Reducing Agent/Reducing Agent (20X)	500 µL	-20°C	-20°C
1000X DAPI/Total DNA Stain (1000X)	20 µL	-20°C	-20°C

3. Materials Required, Not Supplied

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

- Tissue culture vessels and appropriate culturing media; flow cytometry vessels.
- Phosphate Buffered Saline (PBS, pH 7.4).
- Sterile 0.1% Gelatin Solution (optional, only required for adhering suspension cells to the surface).
- Flow cytometer equipped with laser capable of excitation at 488 and 530/590 nm emission filters respectively.
- Fluorescence microscope capable of excitation and emission at 440/490 and 540/580 nm respectively.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 GCK Assay Buffer/Wash Buffer (10X)

1. Dilute the 10X stock 1:10 in sterile water. Mix well. Store at 4°C.

5.2 Fixative Solution I/Fixative Solution

1. Ready to use as supplied. After opening store at 4°C protected from light.

5.3 10X Permeabilization Buffer/Permeabilization Buffer (10X)

1. Dilute the 10X stock 1:10 in sterile water. Mix well. Store at 4°C.

5.4 1000X GlcAz Label/GlcAz Label (1000X)

1. Ready to use as supplied.
2. While in use, keep on ice and minimize light exposure.

5.5 100X Copper Reagent/Copper Reagent (100X)

1. Ready to use as supplied.
2. While in use, keep on ice and minimize light exposure.

5.6 100X Fluorescent Alkyne I/Fluorescent Alkyne (100X)

1. Ready to use as supplied.
2. While in use, keep on ice and minimize light exposure.

5.7 20X Reducing Agent/Reducing Agent (20X)

1. Ready to use as supplied.
2. While in use, keep on ice and minimize light exposure.

5.8 1000X DAPI/Total DNA Stain (1000X)

1. Ready to use as supplied.
2. While in use, keep on ice and minimize light exposure.

6. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

Δ Note: This assay was developed with HeLa (adherent) and Jurkat (suspension) cells and can be modified for any cell line. The protocol below refers to a 96-well tissue culture plate format and assay volume is 100 µL; adjust volumes accordingly for other plate formats. Growth conditions, cell number per well and other factors may affect the incorporation rate of the GlcAz Label; therefore, optimize the assay for your cell type. We suggest an initial test of several GlcAz Label concentrations to find best conditions for your experimental design. Avoid stressing the cells by washes or temperature changes prior to incubation with GlcAz Label.

6.1 Labeling with GlcAz Label

1. Seed the cell suspension of desired density directly into tissue culture vessels, or on coverslips for high resolution microscopy.
2. To immobilize suspension cells for microscopy: add 100 µL of 0.1% gelatin solution into each well of a tissue culture plate, tilt the plate to cover the entire well surface and place it in a tissue culture hood for 1 hour. Gently remove the gelatin solution and seed your cells. Allow the cells to recover overnight before the treatment.
3. Next day, remove the media, and replace it with fresh aliquots containing 1X GlcAz Label. Include appropriate controls.
   - Negative control: cells not exposed to the 1X GlcAz Label or treatment.
   - Positive control: Cells incubated with 1X GlcAz Label only.
4. Add treatments and incubate the cells for additional 1-3 days in a 37°C incubator, or for the period of time required by your experimental protocol. For analysis of trafficking and dynamics of cellular glycans take samples during incubation. Do not remove the drug-containing media while incubating with 1X GlcAz Label to avoid potential reversibility of drug action on label incorporation.

5. Terminate the experiment, remove the media and rinse the cells once with 100 µL of PBS, discard the supernatant. Always pellet the suspension cells at 300 x *g* for 5 minutes throughout the entire protocol. For immobilized suspension cells: Centrifuge the plate at 300 x *g* (or the lowest centrifuge setting) for 5 minutes to gently deposit the cells onto the surface. Tilt the plate and gently remove the media with a pipette tip. It is important to avoid excessive centrifugation speeds, which can damage the cells.

Δ Note: Make note of the place that is used, and perform subsequent aspirations from the same place.

6.2 Fixation and Permeabilization

1. For adherent and suspension cells: Add 100 µL of Fixative Solution I/Fixative Solution per well and incubate the cells for 15 minutes at room temperature protected from light. Remove the fixative and wash the cells twice with 100 µL of 1X GCK Assay Buffer/Wash Buffer.
2. Remove the wash and add 100 µL of 1X Permeabilization Buffer per well, incubate the cells for 10 minutes at room temperature. Remove the Permeabilization Buffer and replace it with a 20 µL of fresh aliquot.

6.3 GlcAz reaction and total DNA staining

1. Prepare 1X Reaction cocktail according to the table below. Volumes should be multiplied by number of samples and reagents added in the exact order. Use the reaction cocktail within 15 minutes of preparation.

Δ Note: Cells should be protected from light during and following the Reaction and DNA staining.

Component	Reaction Mix (µL)
PBS	93 µL
Cooper Reagent (100X)	1 µL
100X Fluorescent Alkyne I/Fluorescent Alkyne (100X)	1 µL
20X Reducing Agent/Reducing Agent (20X)	5 µL

2. Add 100 µL of 1X Reaction cocktail to each sample and incubate the cells for 30 minutes at room temperature protected from light. Remove the reaction cocktail and wash cells three times in 100 µL of GCK Assay Buffer/Wash Buffer. Remove the wash and suspend the cells in 100 µL of PBS.

Δ Note: If no DNA staining is desired, proceed to Microscopic or FACS analysis.

3. DNA staining: Prepare 1X dilution of DAPI/Total DNA Stain and add 100 µL per well. Incubate the cells for 20 minutes at room temperature, or refrigerate at 4°C protected from light. Remove the stain solution and replace with 100 µL of PBS.

6.4 Fluorescence Microscope and FACS analysis

1. Fluorescence Microscope analysis: Examine labeled glycoproteins using FITC filter and UV laser for total DNA staining.
2. FACS analysis: Transfer the 100 µL cell suspension into flow cytometry vessels. Analyze samples in FL-1 channel for signal generated by labeled glycoproteins.

7. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

8. Typical Data

Data provided **for demonstration purposes only.**

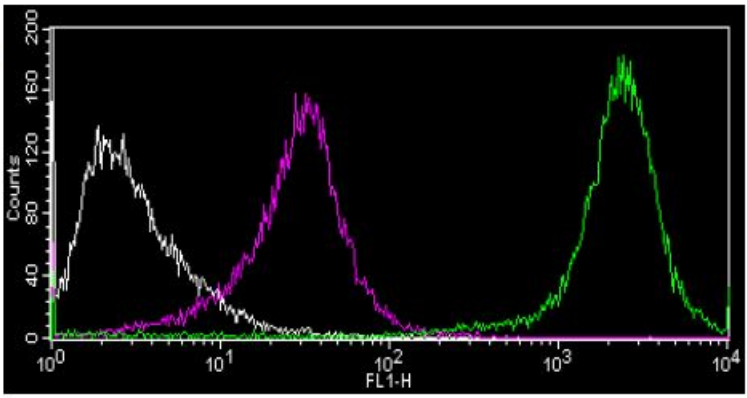

Figure 1. Analysis of metabolic labeling of GlcAz labeled glycans in proliferating cells. Jurkat cells (1 X 10^6 cells/ml) were cultured in presence of 1X GlcAz Label for 24 hours at 37°C. Modified glycoproteins were detected according to the kit protocol and green fluorescence was analyzed by FACS (FL-1 channel). Negative control (white line), Background control (purple line), fluorescence corresponding to intracellular O-GlcNAc-glycosylated proteins (green line).

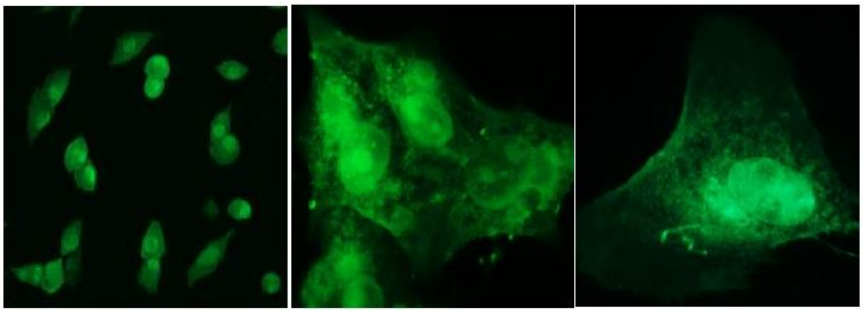

Figure 2. Fluorescence Microscope images of intracellular O-GlcNAc-glycosylated proteins in HeLa cells. High resolution images (middle and right panels) clearly show cytoplasmic and nuclear localization of GlcAz modified glycans.

9. Notes

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)