ab308247 – O-GlcNAc Modified Glycoprotein Assay Kit (FACS/Microscopy, Red)

A highly specific, simple and robust assay for labeling and detection of O-GlcNAc modified glycoproteins.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

Upon arrival, store the entire kit at -20 °C protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

Materials Supplied

Item	Quantity	Storage Condition
Wash Buffer (10X)	25 ml	4 °C
Fixative Solution	10 ml	-20 °C
Permeabilization Buffer (10X)	25 ml	4 °C
GlcAz Label (1000X)	10 µl	-20 °C
Copper Reagent (100X)	100 µl	-20 °C
Fluorescent Alkyne (100X)(Avoid light)	100 µl	-20 °C
Reducing Agent (20X) (Avoid light)	500 µl	-20 °C
Total DNA Stain (1000X) (Avoid light)	20 µl	-20 °C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize 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 suspension cells)
- Flow cytometer equipped with laser capable of excitation at 540/580 nm wavelength (FL-2)
- Fluorescence microscope capable of excitation and emission at 440/490 and 540/580 nm.

Reagent Preparation

Wash Buffer (10X) and Permeabilization Buffer (10X): Thaw at 37 °C to dissolve completely. Dilute the 10X stocks at 1:10 dilution in sterile water and mix well. Store at 4 °C.

Fixative Solution: Ready to use. Divide into aliquots and store at -20 °C, protected from light.

Remaining components: Store at -20 °C, protected from light. While in use, keep on ice and minimize light exposure.

O-GlcNAc Modified Glycoprotein Assay Protocol:

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 with an assay volume of 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 depending on your cell type. We suggest an initial test of several GlcAz Label concentrations for your experimental design. Avoid stressing the cells by washes or temperature changes prior to incubation with GlcAz Label. All steps should be carried out at room temperature (RT) unless otherwise specified. Equilibrate all buffers to RT prior to the experiment.*

Labeling with GlcAz Label:

a. Seed the cell suspension of desired density directly into the tissue culture vessels, or on coverslips for high resolution microscopy. To immobilize the 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 hr. Gently, remove the gelatin solution and seed the cells. Allow the cells to recover overnight before the treatment.

b. Next day, remove the media, and replace it with fresh aliquots containing 1X GlcAz Label. Include the appropriate controls: Negative Control: Unstained cells. i.e., cells not exposed to GlcAz Label or any treatments. Background Control: Cells are not exposed to GlcAz Label and contains only Reaction. Positive Control: Cells are incubated with 1X GlcAz Label only.

c. Add the treatment and incubate the cells for an additional 1-3 days in a 37 °C incubator, or for the period of time as 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.

d. To terminate the experiment, for adherent cells: Remove the media and rinse the cells once with 100 µl of 1X PBS, discard the supernatant. For immobilized suspension cells: Centrifuge the plate at 500 x g (or the lowest centrifuge setting) for 5 min 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. Make note of the position in the well that is used for aspiration and perform subsequent aspirations from the same location. Proceed to the Fixation and Permeabilization step.

Fixation and Permeabilization:

For adherent and suspension cells:

Add 100 µl of Fixative Solution to each well and incubate the cells for 15 min at RT, protected from light. Remove the fixative and wash the cells twice with 100 µl of 1X Wash Buffer. Remove the wash and add 100 µl of 1X Permeabilization Buffer per well. Incubate the cells for 10 min at RT. Remove the Permeabilization Buffer and proceed to reaction and total DNA staining.

GlcAz Reaction and Total DNA Staining:

a. Reaction Cocktail:

Prepare 1X Reaction Cocktail according to the table below. Volumes should be multiplied by number of samples and reagents and added in the order listed below. Use the Reaction Cocktail within 15 min of preparation. *Cells should be protected from light during, and after the reaction and DNA staining.*

	Amount per reaction
PBS	93 µl
Copper Reagent (100X)	1 µl
Fluorescent Alkyne (100X)	1 µl
Reducing Agent (20X)	5µl

b. Reaction:

For Negative Control

Add 100 µl of 1X PBS. For Background Control, Positive Control Cells and Experimental Cells: Add 100 µl of 1X Reaction Cocktail to each sample and incubate the cells for 30 min at RT,

protected from light. Remove the Reaction Cocktail and wash cells three times in 100 µl of 1X Wash Buffer. Remove the final 1X wash and suspend the cells in 100 µl of 1X PBS. Proceed to DNA staining. If no DNA staining is desired, proceed to microscopic or FACS analysis. DNA Staining: Prepare 1X dilution of Total DNA Stain and add 100 µl per well. Incubate the cells for 20 min at RT, or refrigerate at 4 °C, protected from light. Remove the DNA staining solution, wash the cells once with 100 µl PBS.

Δ Note: *Cells are compatible with all methods of slide preparation including wet mount or prepared mounting media.*

Fluorescence Microscope Analysis:

Examine the samples for red fluorescence generated by labeled GlcAz and blue fluorescence for nuclear DNA. FACS Analysis: Harvest the cells by any preferred method and wash with 0.5 ml of ice-cold PBS. Re-suspend the pellets in 100 µl of ice-cold PBS and analyse samples for red fluorescence generated by GlcAz addition during reaction.

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)