

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

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Overview

Product name	O-GlcNAc Modified Glycoprotein Assay Kit (FACS/Microscopy, Green Fluorescence)
Sample type	Adherent cells, Suspension cells
Product overview	<p>The O-GlcNAc Modified Glycoprotein Assay Kit (FACS/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.</p>
Notes	<p>This product is manufactured by BioVision, an Abcam company and was previously called K714 EZClick™ O-GlcNAc Modified Glycoprotein Assay Kit (FACS/Microscopy, Green Fluorescence). K714-100 is the same size as the 100 test size of ab235633.</p> <p>Glycans are vital components of glycoproteins, glycolipids, and proteoglycans in all domains of life. Glycoproteins are grouped by the type of carbohydrate and amino acid linkage site. <i>N</i>-linked glycosylation is a modification of asparagine, whereas <i>O</i>-linked glycosylation occurs through the hydroxyl group of serine and threonine residues. Glycosylation occurs co- or post-translationally on >50% of eukaryotic proteins resulting in membrane-associated, intracellular, or secreted glycoproteins that are crucial in cellular processes, protein bioactivity and metabolic turnover. Modification by <i>O</i>-linked-<i>N</i>-acetyl glucosamine (<i>O</i>-GlcNAc) has rapidly emerged as a major cellular signaling mechanism with number of modified targets similar to protein phosphorylation. Many oncogenes and tumor suppressors are regulated by <i>O</i>-GlcNAcylation. <i>O</i>-GlcNAc modification is ubiquitous among eukaryotes, from yeast to humans and its modifying enzymes have been well characterized. <i>O</i>-GlcNAc modified nuclear and cytosolic targets include: transcription factors, signaling proteins, metabolic enzymes, mitochondrial trafficking, cell cycle regulation, glucose homeostasis. <i>O</i>-GlcNAc glycosylation is implicated in normal brain functions, etiology of neurodegeneration, type II diabetes, and pathways involved in morphogenesis and virulence factors of microbes and plant host cells. Since glycoproteins are not directly encoded in the genome, methods of characterization and analyses of glycoproteins are of great interest.</p>
Platform	Flow cytometer, Fluorescence microscope

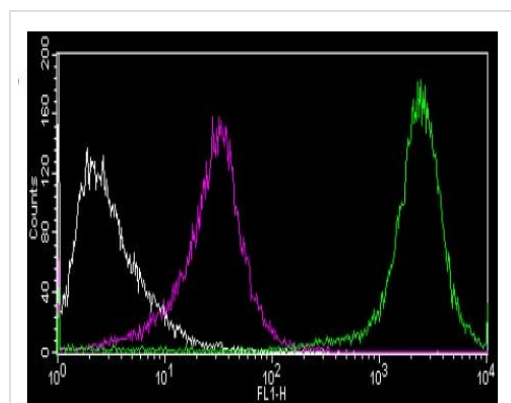
Properties

Storage instructions

Store at -20°C. Please refer to protocols.

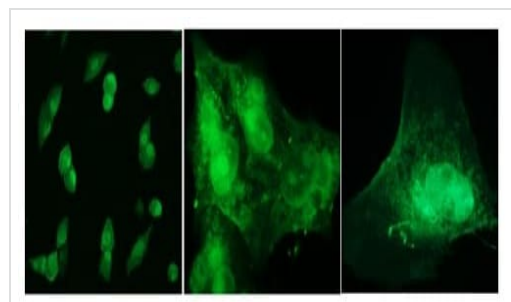
Components	100 tests
Copper Reagent (100X)	1 x 100µl
Fixative Solution	1 x 10ml
Fluorescent Alkyne (100X)	1 x 100µl
GlcAz Label (1000X)	1 x 10µl
Permeabilization Buffer (10X)	1 x 25ml
Reducing Agent (20X)	1 x 500µl
Total DNA Stain (1000X)	1 x 20µl
Wash Buffer (10X)	1 x 25ml

Images



Analysis of metabolic labeling of GlcAz labeled glycans in proliferating cells

Jurkat cells (1×10^6 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).



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.

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