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EZClick™ Sialic Acid (ManAz) Modified Glycoprotein Assay Kit (FACS/Microscopy, Green)

rev 07/20

(Catalog # K441-100; 100 assays; Store at -20°C)

I. Introduction:

Glycans are vital components of glycoproteins, glycolipids, and proteoglycans in all domains of life. 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. Intracellular glycans mediate protein folding, stability, and trafficking while at the cell surface, they participate in recognition, cell-cell interactions involved in adhesion, migration, and embryonic development; host-pathogen interactions critical for bacterial and viral infections; and initiation of immune response. Aberrant glycosylation profiles correlate with inflammation and are universal feature of cancer, with sialic acids playing an especially prominent role as tumor associated carbohydrate antigens (TACAs). Altered sialylation of tumor cell surfaces is associated with several critical malignant properties that include invasiveness and metastatic potential suggesting its implication in clinical diagnosis. Since glycoproteins are not directly encoded in the genome, methods of characterization and analyses of glycoproteins are of great interest. Thus BioVision offers EZClick™ Sialic Acid (ManAz) Modified Glycoprotein Assay Kit, a highly specific, simple and robust method for labeling and detection of *N*-linked glycosylation of cell surface proteins. We use a modified mannosamine precursor that is fed directly into the cells, converted to sialic acid by the sialic acid biosynthetic machinery, and transported to the Golgi apparatus for glycan elaboration. 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. We provide sufficient materials for 100 assays in a 96-well plate format.

II. Applications:

- Identification and characterization of cell surface sialic acid-modified glycoproteins
- Imaging the localization, trafficking, and dynamics of modified glycans
- Detection and quantification of biosynthesis, subcellular localization and turnover of modified glycans
- Screening for genotoxic compounds and effectors of modified glycans in proliferating cells
- Evaluating effects of anti-cancer drugs and genotoxic agents on modified glycans

III. Sample Type:

- Suspension or adherent cell cultures

IV. Kit Contents:

Components	K441-100	Cap Code	Part Number
EZClick™ Wash Buffer (10X)	25 ml	NM	K441-100-1
Fixative Solution	10 ml	WM	K441-100-2
Permeabilization Buffer (10X)	25 ml	NM/Blue	K441-100-3
EZClick™ ManAz Label (1000X)	10 µl	Clear	K441-100-4
Copper Reagent (100X)	100 µl	Blue/Clear Vial	K441-100-5
EZClick™ Fluorescent Alkyne (100X)	100 µl	Green	K441-100-6
Reducing Agent (20X)	500 µl	Yellow	K441-100-7
EZClick™ Total DNA Stain (1000X)	20 µl	Blue/Amber Vial	K441-100-8

V. User Supplied Reagents and Equipment:

- 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

VI. Storage Conditions and Reagent Preparation:

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.

- **10X Wash Buffer and 10X Permeabilization Buffers: Thaw at 37°C to dissolve completely.** Dilute the 10X stocks 1:10 in sterile water, mix well. Store at 4 °C.
- **Fixative Solution: 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.

VII. Assay Protocol:

Notes:

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 ManAz Label; therefore optimize the assay for your cell type. We suggest an initial test of several ManAz Label concentrations to find best conditions for your experimental design. Avoid stressing the cells by washes or temperature changes prior to incubation with ManAz Label. All steps should be carried out at room temperature (RT) unless otherwise specified; equilibrate all buffers to RT prior to the experiment

1. Labeling with ManAz Label:

a. Seed the cell suspension of desired density directly into tissue culture vessels, or on coverslips for high resolution microscopy. **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.

b. Next day, remove the media, and replace it with fresh aliquots containing EZClick™ ManAz Label diluted to 1X. Include appropriate controls. **Negative control** -cells not exposed to the 1X ManAz Label or treatment, **positive control** -cells incubated with 1X ManAz Label only. *Do not add the* EZClick™ ManAz Label *into the* ***negative control*** *cells*.

c. 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 ManAz Label to avoid potential reversibility of drug action on label incorporation.

d. 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 500 x g for 5 min throughout the entire protocol. **For immobilized suspension cells**: Centrifuge the plate at 500 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. *Make note of the place that is used, and perform subsequent aspirations from the same place*. Proceed to the Fixation and Permeabilization.

2. Fixation and Permeabilization:

Optional: If only cell surface glycans are to be stained, Fixation and/or Permeabilization steps might be omitted or modified as they may decrease the availability of surface glycans. Cell pellets should be re-suspended in 20 µl PBS prior click reaction.

For adherent and suspension cells: Add 100 µl of Fixative Solution per well and incubate the cells for 15 min at RT protected from light, remove the fixative. Wash the cells twice with 100 µl of 1X Wash Buffer. Remove the wash and add 100 µl of 1X Permeabilization Buffer per well and incubate the cells for 10 min at RT. Remove the Permeabilization Buffer. Proceed to EZClick™ reaction and total DNA staining.

3. EZClick™ ManAz reaction and total DNA staining:

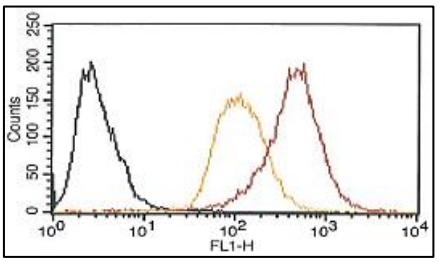
a. Reaction cocktail: Prepare 1X EZClick™ reaction cocktail according to the table below. Volumes should be multiplied by number of samples and reagents added in the exact order. Use the reaction cocktail within 15 minutes of preparation. *Cells should be protected from light during, and following the EZClick™ reaction and DNA staining*.

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

b. EZClick™ Reaction: For Negative Control Cells: add 100 µl of 1X PBS. For Positive Control Cells and Experimental Cells: add 100 µl of 1X EZClick™ Reaction cocktail to each sample and incubate the cells for 30 min at room temperature protected from light. Remove the reaction cocktail and wash cells three times in 100 µl of Wash Buffer. Remove the wash and suspend the cells in 100 µl of PBS. Proceed to DNA staining. If no DNA staining is desired, proceed to Microscopic or FACS analysis. **DNA staining:** Prepare 1X dilution of EZClick™ 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.

4. Fluorescence Microscope analysis: Examine labeled glycoproteins using FITC filter and UV laser for total DNA staining.
FACS analysis: Transfer the 100 µl cell suspension into flow cytometry vessels. Analyze samples in FL-1 channel for signal generated by labeled glycoproteins. **Note:** If analyzing cell surface proteins, avoid trypsin to detach cells as surface proteins might be cleaved.

A.



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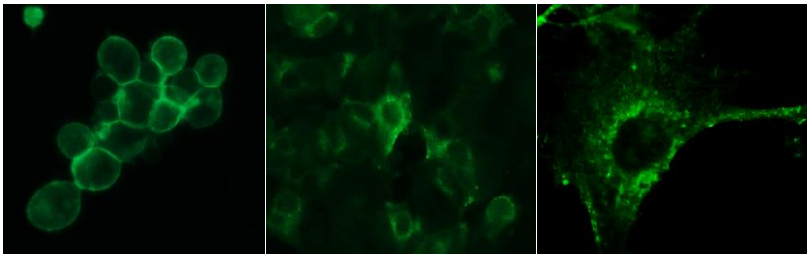

Figures: Analysis of metabolic labeling of ManAz labeled glycans in proliferating cells. **(A)** Jurkat cells (1X10^6 cells/ml) were cultured in presence of 1X EZClick™ ManAz Label for 24 hours at 37°C. Modified glycoproteins were detected according to the kit protocol and green fluorescence was analyzed by FACS (FL-1 channel). Fluorescence in fixed and permeabilized cells **(brown line)** corresponds to surface and intracellular glycans whereas decreased signal in unprocessed cells resembles surface cell glycans **(yellow line)**; negative control **(black line)**. **(B)** Fluorescence Microscope analysis of cell surface glycans in non-fixed and non-permeabilized Jurkat cells **(middle panel)** and subcellular localization of ManAz modified glycans in fixed and permeabilized HeLa cells **(middle panel)**. High resolution image (right panel) shows transported to the Golgi apparatus labeled glycans.

VIII. RELATED PRODUCTS:

EZClick™ Global Protein Synthesis Assay Kit (FACS/Microscopy), Red Fluorescence (K715)
EZClick™ Global Phospholipid Synthesis Assay Kit (FACS/Microscopy), Red Fluorescence (K717)
EZClick™ O-GalNAc Modified Glycoprotein Assay Kit (FACS/Microscopy, Green Fluorescence) (K714)
EZClick™ O-GlcNAc Modified Glycoprotein Assay Kit (FACS/Microscopy, Green Fluorescence) (K560)

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