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ab241020 Fucose (FucAz) Modified Glycoprotein Assay Kit

View Fucose (FucAz) Modified Glycoprotein Assay Kit datasheet: www.abcam.com/ab241020

For the measurement of fucose-containing glycoproteins in suspension and adherent cells.

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

Fucose (FucAz) Modified Glycoprotein Assay Kit (ab241020) is a highly specific, simple and robust method for labeling and detection of fucosylated proteins within cells. It uses a fucose analog that is fed directly into the cells, processed via the fucose salvage pathway and incorporated into the glycoproteins. This is followed by click reaction with an alkyne-containing dye so this system offers a powerful method for imaging the localization, trafficking, and dynamics of glycans, or detection by FACS for quantitative studies.

Fucose-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. The kit provides sufficient materials for 100 assays in a 96-well plate format.

2. Protocol Summary

Culture suspension or adherent cells.

Seed cells at the desired density into tissue culture vessels, or coverslips for high resolution microscopy (treat coverslips with gelatin for suspension cells).

Next day label cells with FucAz Label for 1-3 days at 37°C (do not label Negative Control cells). Include any test compounds throughout the full incubation period.

Remove FucAz Label-containing media, wash and fix cells with Fixative Solution I/Fixative Solution at room temperature.

Incubate cells with freshly prepared Reaction Cocktail for 30 minutes at room temperature, protected from light. Stain DNA with DAPI/Total DNA Stain, if required.

Analyse cells by fluorescence microscopy (FITC filter for labeled glycoproteins, UV laser for DNA) or FACS (FL-1 channel for labeled glycoproteins).

3. 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.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
10X Wash Buffer IV/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 FucAz Label/FucAz 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

4. Materials Required, Not Supplied

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

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

5. 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.

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 10X Wash Buffer IV/Wash Buffer (10X)

Thaw at 37 °C to dissolve completely. Dilute the 10X stock 1:10 in sterile water, mix well. Store at 4 °C. Warm to room temperature prior to use.

6.2 Fixative Solution I/Fixative Solution

Divide into aliquots and store at -20 °C protected from light. Ready to use as supplied. Warm to room temperature prior to use.

6.3 10X Permeabilization Buffer/Permeabilization Buffer (10X)

Thaw at 37 °C to dissolve completely. Dilute the 10X stock 1:10 in sterile water, mix well. Store at 4 °C. Warm to room temperature prior to use.

6.4 1000X FucAz Label/FucAz Label (1000X)

Ready to use as supplied. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.5 Copper Reagent

Ready to use as supplied. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.6 Fluorescent Alkyne I/Fluorescent Alkyne

Ready to use as supplied. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.7 Reducing Reagent

Ready to use as supplied. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.8 DAPI/Total DNA Stain

Ready to use as supplied. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

7. Assay Procedure

General sample information:

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

7.1 Labeling with FucAz Label:

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

   ΔNote: 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.

2. Next day, remove the media, and replace it with fresh aliquots containing 1X FucAz Label. Include appropriate controls.
   Negative control: cells not exposed to the 1X FucAz Label or treatment.
   Positive control: cells incubated with 1X FucAz Label only.

   ΔNote: Do not add the FucAz Label into the negative control cells.

3. 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

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

4. 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. Make a note of the place that is used, and perform subsequent aspirations from the same place.
   Proceed to the Fixation and Permeabilization steps.

7.2 Fixation and Permeabilization:

1. For adherent 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 200 µL of 1X Wash Buffer IV/Wash Buffer. 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 fresh aliquot. Proceed to FucAZ labeling reaction and total DNA staining.
2. For suspension cells: Resuspend the cells in 100 µL of Fixative Solution I/Fixative Solution and incubate the cells for 15 minutes at room temperature, protected from light. Centrifuge cells at 900 x g for 5 minutes and remove the fixative. Wash the cells once with 0.5 mL of 1X Wash Buffer IV/Wash Buffer. Remove the fixative and wash the cells twice with 100 µL of 1X Wash Buffer IV/Wash Buffer. Centrifuge cells at 900 x g for 5 minutes and remove the supernatant and resuspend the cells in 100 µL of 1X Permeabilization Buffer. Incubate the cells for 10 minutes at room temperature. Centrifuge cells at 900 x g for 5 minutes and remove the Permeabilization Buffer. Proceed to FucAZ labeling reaction and total DNA staining.

7.3 FucAz labeling and DNA staining:

1. Prepare a Reaction Cocktail as detailed in the table:

   Volumes should be multiplied by number of samples and reagents added in the exact order. Use the reaction cocktail within 15 minutes of preparation.
   ΔNote: Cells should be protected from light during, and following the FucAz labeling reaction and DNA staining.

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

2. FucAz labeling reaction: Add 100 µL of 1X Reaction Cocktail to each sample and incubate the cells for 30 minutes at room temperature, protected from light. Centrifuge cells at 900 x g for 5 minutes. Remove the reaction cocktail and wash cells three times in 100 µL of Wash Buffer IV/Wash Buffer. Remove the wash buffer and suspend the cells in 100 µL of PBS. Proceed to DNA staining, if required. 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. Centrifuge cells at 900 x g for 5 minutes, remove the stain solution, and replace with 100 µL of PBS.

7.4 Fluorescence microscope analysis:

Examine labeled glycoproteins using FITC filter and UV laser for total DNA staining.

7.5 FACS analysis:

Harvest the adherent cells by preferred method; wash with 100 µL of PBS. Centrifuge cells at 900 x g for 5 minutes. Re-suspend the cell pellets in 100 µL of ice-cold PBS and transfer the cell suspension into flow cytometry vessels. Analyze samples in FL-1 channel for signal generated by labeled glycoproteins.

8. Typical Data

Data provided **for demonstration purposes only.**

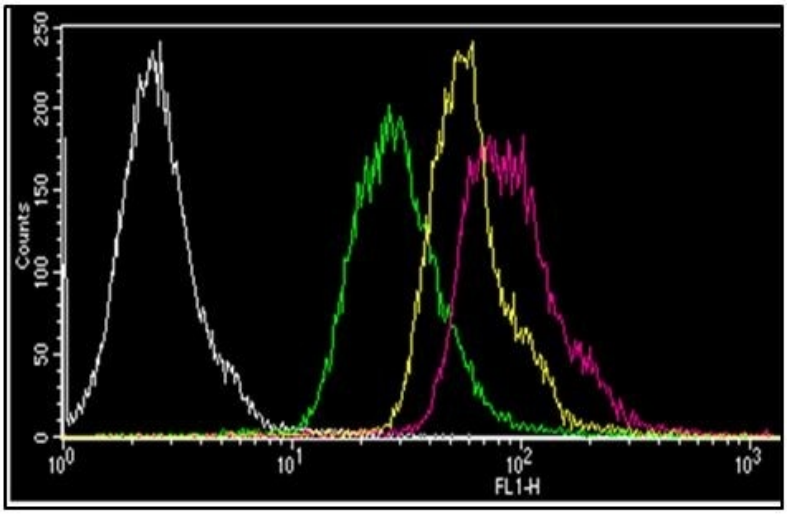

Figure 1. Analysis of metabolic labeling of FucAz labeled glycans in proliferating cells. Jurkat cells (1 x 10^6 cells/mL) were cultured in presence of 1X FucAz Label for 72 hours at 37°C. Modified glycoproteins were detected according to the kit protocol and green fluorescence was analyzed by FACS (FL-1 channel). Negative Control (white line), Background Control (green line), Positive Control - fluorescence corresponding to fucosylated glycoproteins (pink line), Tunicamycin-induced suppression of fucosylation (yellow line.

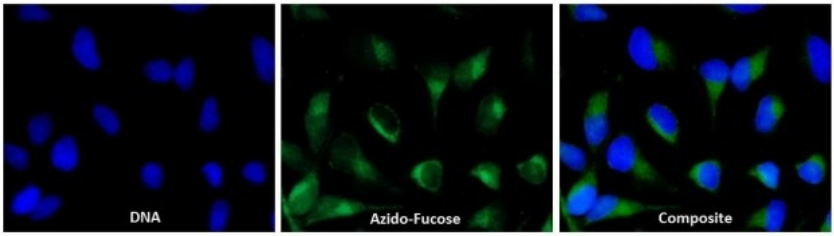

Figure 2. Fluorescence Microscope images of subcellular localization of Fucosylated glycoproteins (middle panel, green)in fixed HeLa cells. Nuclear staining (left panel) and composite image (right panel).

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)