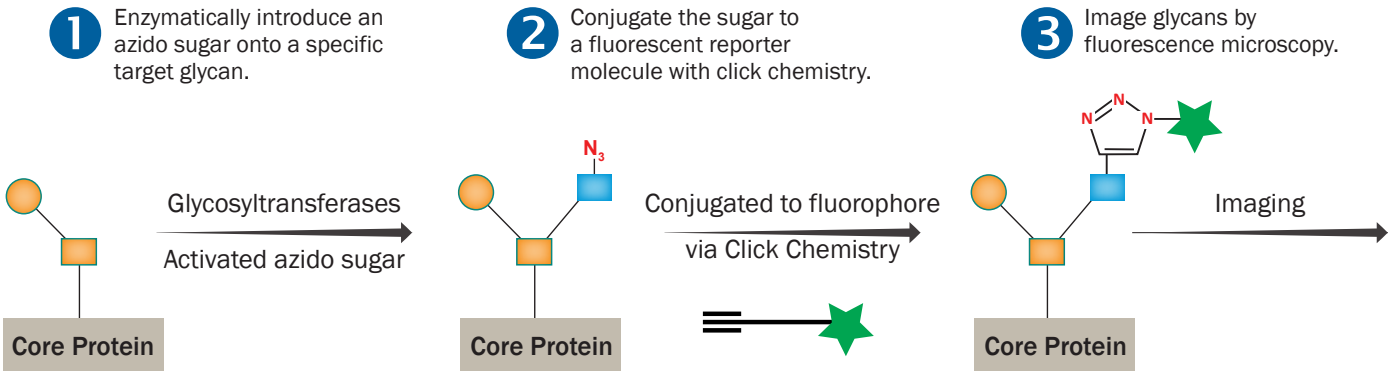
APPLICATION NOTE

Protocol for Fluorescence Imaging of O-GlcNAc

Our technology takes advantage of the selective action of glycosyltransferases on substrate glycans to provide the most specific glycan visualization assay on the market. Protocols are guidelines. Parameters need to be optimized by end users.

Read the entire document before beginning your experiment.

Assay Principle



Download our poster for more information!

Reagents Required

Reagents required from R&D Systems

- OGT
- OGA
- B3GALNT2
- UDP-Azido-GalNAc
- UDP-Azido-GlcNAc
- Biotinylated Alkyne

Reagents required from a third-party vendor

- MES
- Triton® X-100
- $MgCl_2$
- $MnCl_2$
- $CaCl_2$
- Cu^{2+}
- BSA
- NaCl
- Ascorbic Acid
- UDP-GlcNAc
- Streptavidin, Alexa-Fluor
- Fluorescence Microscope

Stock Solutions

- Cu^{2+}, 1 mM in deionized water
- Ascorbic Acid, 20 mM in deionized water

R&D SYSTEMS
a bio-techne® brand

Assay Procedure

1. Culture cells according to standard protocols.
2. Design and execute planned experiments.
3. Rinse cells with sterile phosphate buffered saline (PBS) 3–5 times.
4. Fix cells at room temperature with 4% paraformaldehyde in PBS for 30 minutes.
5. Wash cells 5 times with PBS. Cells can be stored in PBS at 4 °C until ready for glycan labeling.
6. **Pretreatment Reactions**

   Skip to step 8 unless you want to install (close a site) or remove (open a site) O-GlcNAc from serine/threonine residues. For detecting existing O-GlcNAc no pretreatment reaction is needed.

   Close an O-GlcNAc site.

   - Use OGT to install GlcNAc on protein serine/threonine residues.
   - Treat cells with 1 mg of OGT in the presence of 50 nmol of UDP-GlcNAc in 50 µL of 25 mM MES pH 7.0, 0.5% (w/v) Triton® X-100, 2.5 mM $MgCl_2$, 10 mM $MnCl_2$, 1.25 mM $CaCl_2$ and 0.75 mg/mL BSA for at 37 °C for 1 hour. This reaction is scaled for 1 well of a 96 well plate.

   Open an O-GlcNAc site.

   - Use OGA to remove GlcNAc from protein serine/threonine residues.
   - Treat cells with 1 mg of OGA in 50 µL of 50 mM MES pH 5.5, 150 mM NaCl, 0.5% Triton® X-100 at 37 °C for 1 hour. This reaction is scaled for 1 well of a 96 well plate.
7. Rinse cells with PBS 3–5 times.
8. **Incorporation Reaction**

   The following labeling buffer is used: 25 mM MES pH 7.0, 0.5% (w/v) Triton® X-100, 2.5 mM $MgCl_2$, 10 mM $MnCl_2$, 1.25 mM $CaCl_2$ and 0.75 mg/mL BSA. This reaction is scaled for 1 well of a 96 well plate.

   - For imaging **closed O-GlcNAc sites**, incubate cells with 50 µL labeling buffer supplemented with 2 nmol of UDP-GalNAz and 1 µg of B3GALNT2 at 37 °C for 30 minutes.
   - For imaging **open O-GlcNAc sites**, incubate cells in 50 µL labeling buffer supplemented with 2 nmol of UDP-GlcNAz and 1 mg of OGT at 37 °C for 30 minutes.
9. Rinse cells with PBS 3–5 times.

Additional Notes

1. Parameters need to be optimized by end users.
2. Adherent cells show better staining and are less likely to wash away during processing.
3. Non-adherent cells may be labeled using microcentrifuge tubes.
4. Fixation may result in decreased signal. Glycans can also be labeled in live cells. For cell membrane glycans or extracellular matrices, omit Triton®X-100 in the reaction buffer and fix with paraformaldehyde after the pretreatment reaction.
5. The protocol on the left is for a single well (50 µl) of cells in a 96-well plate. For staining cells other than on 96-well plate, adjust the reaction volume, enzyme, and substrate inputs proportionally according to the size of wells.
6. Use negative controls (no labeling enzyme) to reveal non-specific staining due to the click chemistry reaction or endogenous biotin containing molecules.

Customer Support

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