Click-iT™ *O*-GlcNAc Enzymatic Labeling System

Catalog Numbers C33368, C33370, C33372

Pub. No. MAN0002360 Rev. B

Product description

- The Click-iT™ *O*-GlcNAc Enzymatic Labeling System provides a highly sensitive and efficient method for the *in vitro* modification of *O*-GlcNAc modified proteins (figure 1).
- The *O*-GlcNAc (*O*-linked *N*-acetylglucosamine) modification is a highly dynamic intracellular regulatory modification or process similar to phosphorylation, which dramatically alters the posttranslational functions of targeted proteins. This modification is ubiquitously present across all eukaryotes, ranging from yeast to humans, and the enzymes responsible for its dynamic addition and removal have been thoroughly characterized.
- Proteins are enzymatically labeled using the permissive mutant β-1,4-galactosyltransferase (Gal-T1 (Y289L)) (figure 2), which transfers azido-modified galactose (GalNAz) from UDP-GalNAz to *O*-GlcNAc residues on the target proteins.
- Target proteins can then be detected using one of the Click-iT™ Protein Analysis Detection Kits listed in Table 2, which are compatible with downstream mass spectrometry (MS) analyses including LC-MS/MS and MALDI MS, and Invitrogen™'s Multiplexed Proteomics™ technologies (Table 2).
- The labeling and detection process can be accomplished in under 24 hours and is highly sensitive, detecting as little as 0.04 pmole IgG, proteins which contain 0–4 available terminal GlcNAc (figure 3).

Contents and storage

Table 1 Click-iT™ *O*-GlcNAc Enzymatic Labeling System contents and storage

Material	Amount	Concentration	Storage and Stability [1]
UDP-GalNAz (Component A)	50 µg	N/A	• 2–8°C • Desiccate
Gal-T1 (Y289L) (Component B)	125 µL	N/A	• 2–8°C • Do not freeze
Click-iT™ *O*-GlcNAc enzymatic labeling buffer (Component C)	1 mL	2.5X in a solution containing 125 mM NaCl, 50 mM HEPES, 5% NP-40, pH 7.9	• ≤6°C • Avoid freeze-thaw cycles
$MnCl_2$ (Component D)	150 µL	100 mM	2–8°C
Goat anti-mouse IgG positive control protein (Component E)	25 µg	N/A	2–8°C

[1] When stored as directed, the kit is stable for at least 6 months

Required materials not supplied

- 1% SDS in 20 mM HEPES buffer, pH 7.9
- 10 mM HEPES buffer, pH 7.9
- 1% SDS in 50 mM Tris-HCl, pH 8.0
- Methanol
- Chloroform
- 18 megaOhm purified water

Workflow

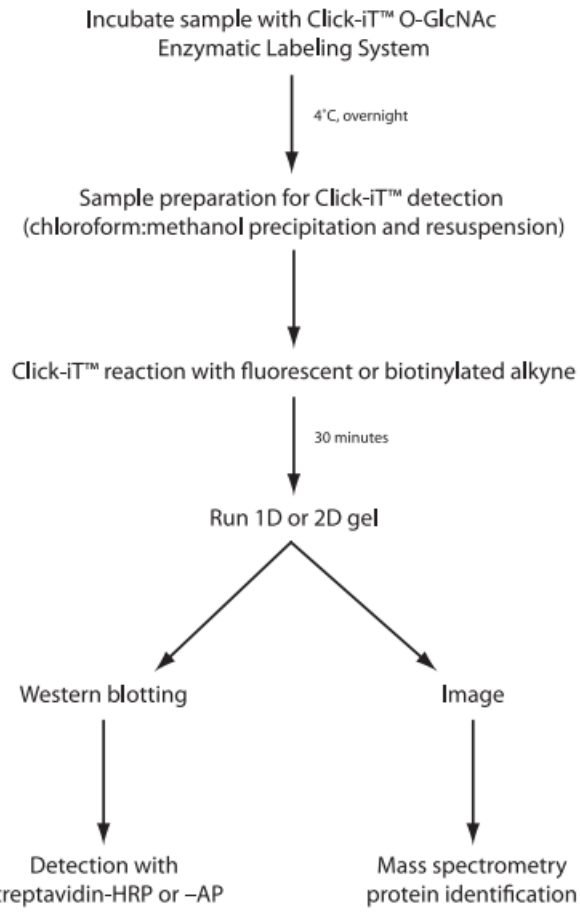

Figure 1 Workflow diagram of the Click-iT™ O-GlcNAc enzymatic labeling and detection scheme

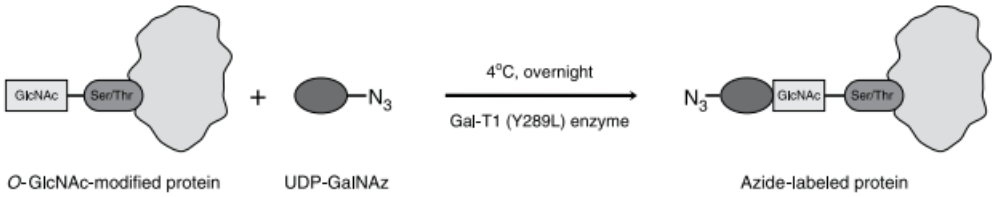

Figure 2 Enzymatic labeling of an *O*-GlcNAc–modified protein with UDP-GalNAz and Gal-T1 (Y289L)

Detection and Multiplexed Proteomics™ compatibility of Click-iT™ protein analysis detection kits

Table 2 Table depicting the compatibility of Click-iT™ protein analysis detection kits

Product	Catalog. no.	Ex/Em [1]	Excitation source	Detection method	Multiplexed Proteomics™ compatibility
Click-iT™ Tetramethylrhodamine (TAMRA) Protein Analysis Detection Kit	C33370	545/580 nm	300 nm UV illumination or 532 nm laser	• 1D or 2D gel • Western blot • Mass spectrometry	• Pro-Q™ Emerald 300 glycoprotein gel stain • SYPRO™ Ruby protein gel stain • Western detection with anti-TAMRA antibody
Click-iT™ Dapoxyl™ Protein Analysis Detection Kit	C33371	370/580 nm	300 or 365 nm UV illumination	• 1D or 2D gel • Mass spectrometry	• Pro-Q™ Diamond phosphoprotein gel stain • SYPRO™ Ruby protein gel stain • Western detection with anti-TAMRA antibody
Click-iT™ Biotin Protein Analysis Detection Kit	C33372	N/A	N/A	• Western blot • Mass spectrometry	Western detection with streptavidin

[1] Excitation and emission maxima in nm.

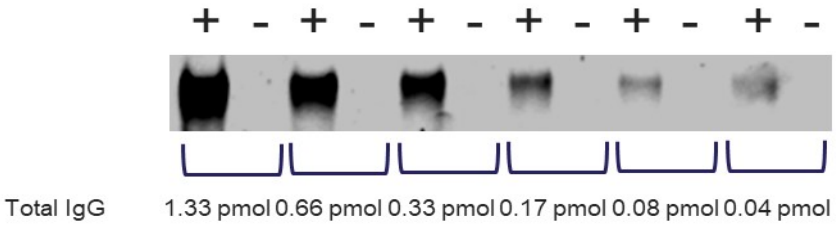

Figure 3 Dilution series of Goat anti-mouse IgG labeled with the Click-iT™ *O*-GlcNAc Enzymatic Labeling System

Detection was performed using the Click-iT™ TAMRA Alkyne Detection Reagent (+). Alternate lanes contain IgG subjected to the same procedure without the addition of the Gal-T1 (Y289L) enzyme (–). IgG antibodies have 0–4 terminal GlcNAc, translating to 40 fmol GlcNAc in the 0.04 pmol band and demonstrating the sensitivity of this detection technology.

Experimental protocol

Note:

- Use soluble protein fractions or subcellular fractions that are free of cell-surface glycoproteins.
- Although cell-surface glycans are present at low levels, they can contain terminal GlcNAc residues that also label with the Gal-T1 (Y289L) enzyme.
- If you include cell-surface glycoproteins in the assay, use PNGase F to cleave cell-surface glycans before labeling with the enzyme, while minimizing false positives from labeling cell-surface glycoproteins.

1 Prepare UDP-GalNAz stock solution

To prepare 0.5 mM solution of UDP-GalNAz, add 144 µL of 10 mM HEPES buffer, pH 7.9, to Component A and mix well to ensure complete reconstitution

Note: Keep the solution on ice while using it. Aliquot and freeze any unused portion at ≤–80°C. Store the stock solution as directed to ensure its stability for up to 1 year.

2 Prepare IgG stock solution

To prepare 1 µg/µL solution of IgG (positive control), add 25 µL 1% SDS,20 mM HEPES, pH 7.9 to Component E. Mix well, then store the unused portion at –20°C.

Note: When stored as directed, this stock solution is stable for up to 1 year.

3 Prepare the sample

1. Aliquot 80–200 µg of lysate, tissue extract, or pure protein into a 1.5 mL microcentrifuge tube.
2. If necessary, adjust the sample volume to 200 µL using 1% SDS, 20 mM HEPES pH 7.9.

3 *(continued)*

3. Precipitate the detergents and proteins using the chloroform/methanol precipitation method below:
   a. Add 600 µL of methanol to the 200 µL sample and briefly vortex the mixture.
   b. Add 150 µL of chloroform and briefly vortex the mixture.
   c. Add 400 µL of 18 megaOhm water and briefly vortex the mixture.
   d. Centrifuge for 5 minutes at 13,000–18,000 × g, then carefully remove and discard as much of the upper aqueous phase as possible while leaving the interface layer containing the protein precipitate intact.
   e. Add 450 µL of methanol to the tube, and briefly vortex the mixture.
4. Centrifuge for 5 minutes at 13,000–18,000 × g to pellet the protein, then remove and discard the supernatant.
5. Leave the cap open, cover the tube with a lint-free tissue, and allow the pellet to air dry for 5 minutes.

   Note: You may air dry the samples longer, up to overnight, but extended drying times can make the protein more difficult to resolubilize.
6. Resuspend the protein in up to 40 µL of 1% SDS in 20 mM HEPES pH 7.9.
7. Heat at 90°C for 5–10 minutes to completely dissolve the proteins.
8. Vortex gently and cool on ice for 3 minutes.

   Note: Longer cooling can cause the SDS to precipitate. If this occurs, warm the sample to room temperature and briefly vortex it to resolubilize the SDS. Visually inspect the sample to ensure the pellet has dissolved.

4 Label the protein sample

1. In a separate 1.5 mL tube, pipet 4 µL of IgG control protein (according to Prepare IgG stock solution).
2. Set up the test reaction and the positive control reaction according to the instructions below.

   Note: Add the various reaction components to each tube in the correct order and perform any necessary mixing steps as specified.
3. To each tube add the following components in order: the 18 megaOhm water, followed by the labeling buffer, and the $MnCl_2$ as described in Table 3.
4. Briefly vortex to mix, and then briefly centrifuge to collect the contents of the tube.
5. Add UDP-GalNAz (according to Prepare UDP-GalNAz stock solution) to each sample. Pipet up and down to mix.
6. Remove 50 µL from the test reaction and pipet into a separate 1.5 mL microcentrifuge tube for use as an unlabeled (negative) control.
7. Add Gal-T1 (Y289L) enzyme (Component B), to each sample. Pipet up and down to mix.
8. Incubate all reactions, including the unlabeled control(s), at 4°C overnight (14 to 24 hours).

4 *(continued)*

9. Store samples at –20˚C until analyzed using the Click-iT™ Protein Analysis Detection Kits.

Table 3 Volumes for Click-iT™ enzymatic labeling reactions:

Reaction components[1]	Test reaction	Positive control reaction
Protein of interest: 2–5 µg/µL in 1% SDS, 20 mM HEPES pH 7.9	40 µL	—
IgG control protein	—	4 µL
18 megaOhm water	49 µL	4.5 µL
Labeling buffer [2]	80 µL	8 µL
$MnCl_2$, 100 mM [3]	11 µL	1.5 µL
UDP-GalNAz	10 µL	1 µL
	(remove 50 µL to a separate tube to serve as negative control)	
Gal-T1 (Y289L) [4]	7.5 µL (to the remainder of the reaction)	1 µL
Final volume	~150 µL (after removing 50 µL for negative control)	20 µL

[1] Mix after the addition of the component(s)
[2] Component C
[3] Component D
[4] Component B

5 Prepare sample for Click-iT™ Protein Analysis Detection

This product does not use azide/alkyne click chemistry, although the following protocol prepares the sample for such a reaction.

1. Chloroform/methanol precipitate the protein sample and negative control proteins, from the reaction mixtures to remove excess UDP-GalNAz (see step 3.2 and step 3.3).

   Note: You do not need to precipitate the IgG positive control reaction; it will be sufficiently diluted in the detection reaction.

2. Air dry the protein sample and negative control pellets for 5 minutes.
3. Resuspend the protein sample and negative control pellets in 50 µL of 1% SDS in 50 mM Tris-HCl, pH 8.0.
4. Add 30 µL of 1% SDS in 50 mM Tris-HCl, pH 8.0 to the IgG positive control reaction.
5. You can now label the samples with any of the Click-iT™ detection reagents.
6. Refer to Table 2 to choose the appropriate Click-iT™ Protein Analysis Detection Kit and Multiplexed Proteomics™ technologies based on the analytical method you plan to use.

Related products

Catalog No.	Product
A6397	**TRITC Polyclonal Antibody**, 500 µL
C10102	**Click-iT™ AHA (L-Azidohomoalanine)**, 5 mg
C21852	**CandyCane™Glycoprotein Molecular Weight Standards**, 400 µL
C33365	**Click-iT™ GalNAz Metabolic Glycoprotein Labeling Reagent (Tetraacetylated *N*-Azidoacetylgalactosamine)**, 5.2 mg
C33366	**Click-iT™ ManNAz Metabolic Glycoprotein Labeling Reagent (tetraacetylated *N*-Azidoacetyl-D-Mannosamine)**, 5.2 mg
C33366	**Click-iT™ GlcNAz Metabolic Glycoprotein Labeling Reagent (tetraacetylated *N*-Azidoacetylglucosamine)**, 5.2 mg
C33370	**Click-iT™ Tetramethylrhodamine (TAMRA) Protein Analysis Detection Kit**, 1 kit
C33372	**Click-iT™ Biotin Protein Analysis Detection Kit**, 1 kit
M33305	**Multiplexed Proteomics™ Phosphoprotein Gel Stain Kits (with 1 L each of Pro-Q™ Diamond and SYPRO™ Ruby Gel Stains)**, 1 set
M33306	**Multiplexed Proteomics™ Phosphoprotein Gel Stain Kits (with 200 mL each of Pro-Q™ Diamond and SYPRO™ Ruby Gel Stains)**, 1 set
P33350	**PeppermintStick™ Phosphoprotein Molecular Weight Standards**, 400 µL
P21855	**Pro-Q™ Emerald 300 Glycoprotein Gel Stain Kit, with SYPRO™ Ruby Protein Gel Stain**, 1 kit
P33300	**Pro-Q™ Diamond Phosphoprotein Gel Stain**, 1 L
P33301	**Pro-Q™ Diamond Phosphoprotein Gel Stain**, 200 mL
P33302	**Pro-Q™ Diamond Phosphoprotein Gel Stain**, 5 L
R33200	**EZQ™ Protein Quantitation Kit**, 2000 Assays
S12000	**SYPRO™ Ruby Protein Gel Stains**, 1 L
S12001	**SYPRO™ Ruby Protein Gel Stains**, 200 mL
S12001	**SYPRO™ Ruby Protein Gel Stains**, 5 L

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 29851 Willow Creek Road | Eugene, Oregon 97402 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0002360 B

Revision	Date	Description
B	9 December 2024	New document created for Click-iT™ *O*-GlcNAc Enzymatic Labeling System in CCMS. Converted the legacy document, to the current document template, with associated updates to the publication number, limited license information, warranty, trademarks, and logos.
A	26 October 2007	Basis for revision