

Click-iT® Protein Reaction Buffer Kit

Catalog no. C10276

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Click-iT® reaction buffer (Component A)	2.6 mL	2X concentrate	<ul style="list-style-type: none"> • ≤25°C • Desiccate 	When stored as directed this kit is stable for at least 6 months.
Copper (II) sulfate (CuSO ₄) (Component B)	270 µL	40 mM aqueous solution		
Click-iT® reaction buffer additive 1 (Component C)	40 mg	Not applicable		
Click-iT® reaction buffer additive 2 (Component D)	30 mg	Not applicable		
Number of assays: Sufficient material is supplied for 25 reactions, based on the protocol below.				

Introduction

Click chemistry describes a powerful new class of chemical reactions that utilize bio-orthogonal or biologically unique moieties to label and detect a molecule of interest using a simple, two-step procedure.¹⁻⁴ In the first step, an azide- or alkyne-containing biomolecule is actively incorporated into the protein (Table 2). Unlike other labels, the azide- and alkyne-tag is small enough that tagged biomolecules (e.g., sugars⁵, and amino acids⁶) are acceptable substrates for the enzymes that incorporate these building blocks into biopolymers such as proteins (Figure 1). The second step or detection step utilizes the chemoselective ligation or “click” reaction between an azide and an alkyne where the modified protein is detected with the corresponding azide- or alkyne-containing dye or hapten (Figure 2, Table 3).

The Click-iT® Protein Reaction Buffer Kit includes everything required to perform the click reaction on proteins labeled with an azide- or alkyne-tagged biomolecule and the corresponding click detection reagent for subsequent standard protein biochemical analyses (e.g., western blots, mass spectrometry). Although a variety of reagents are available for performing click-based detection reactions, currently only tetramethylrhodamine and biotin are recommended as the detection reagents for protein analyses as they do not noticeably affect protein migration. Detection sensitivity in one dimensional (1D) gels and western blots has been found to be in the low femtomole range and compatible with downstream LC-MS/MS and MALDI-MS analysis.

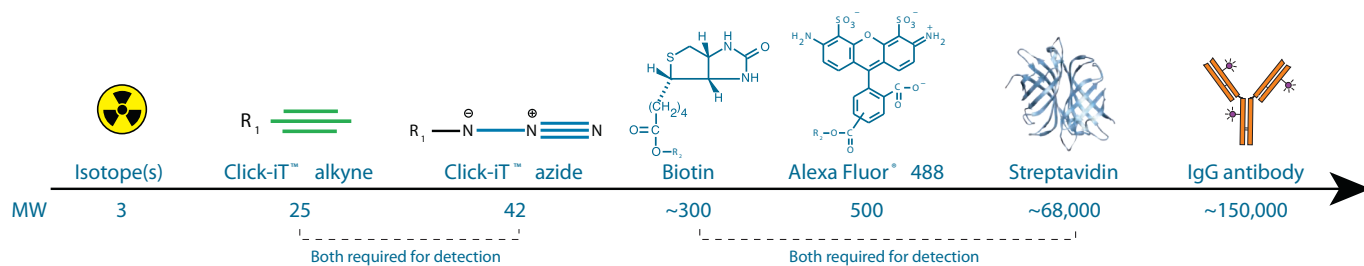


Figure 1. Relative size of detection molecules commonly used in cellular analysis.

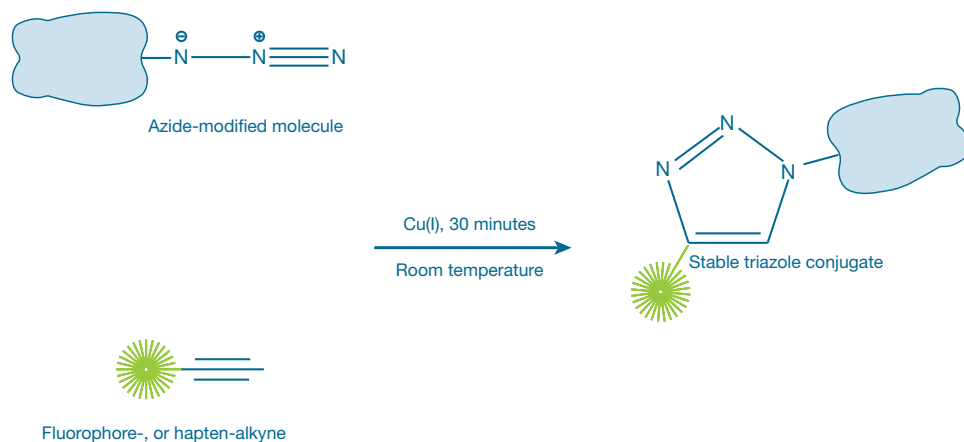


Figure 2. Click azide/alkyne reaction. The azide and alkyne moieties are interchangeable, whereupon the molecule can be labeled with an alkyne and react with a fluorophore- or hapten-azide.

Table 2. Alkyne and azide-modified compounds for labeling proteins.

Compound	Application	Cat. no.	Azide or alkyne
Click-iT® GalNAz (tetraacetylated <i>N</i> -azidoacetylgalactosamine)	<i>O</i> -Linked glycoproteins	C33365	azide
Click-iT® GlcNAz (tetraacetylated <i>N</i> -azidoacetylglucosamine)	<i>O</i> -GlcNAc-modified glycoproteins	C33367	azide
UDP-GalNAz	<i>O</i> -GlcNAc-modified glycoproteins*	C33368	azide
Click-iT® ManNAz (tetraacetylated <i>N</i> -azidoacetyl-D-mannosamine)	Sialic acid-modified proteins	C33366	azide
Click-iT® HPG (L-homopropargylglycine)	Nascent protein synthesis	C10186	alkyne
Click-iT® AHA (L-azidohomoalanine)	Nascent protein synthesis	C10102	azide
Click-iT® palmitic acid, azide	Palmitoylated proteins	C10265	azide
Click-iT® myristic acid, azide	Myristoylated proteins	C10268	azide
Click-iT® farnesyl alcohol, azide	Farnesylated proteins	C10248	azide
Click-iT® geranylgeranyl alcohol, azide	Geranylgeranylated proteins	C10249	azide
Click-iT® fucose alkyne (tetraacetylfucose alkyne)	Fucosylated glycans	C10264	alkyne

**O*-GlcNAc labeled proteins can also be tagged with an azide for subsequent detection and analysis with alkyne-tagged detection reagents using UDP-GalNAz in the Click-iT® *O*-GlcNAc Enzymatic Labeling System (Cat. no. C33368).

Table 3. Recommended azide- and alkyne-containing detection reagents for protein analyses*.

Label	Azide or alkyne	Cat. no.	Ex/Em†	Detection technique
Tetramethylrhodamine	azide	T10182	545/580	<ul style="list-style-type: none"> • 1D or 2D gel electrophoresis • Western blot with an anti-TAMRA antibody • Mass spectrometry (MS)
	alkyne	T10183		
Biotin	azide	B10184	Not applicable	<ul style="list-style-type: none"> • Western blot with a streptavidin conjugate • MS
	alkyne	B10185		

*Although other azide- and alkyne-tagged detection reagents are available, they are not recommended for these detection techniques. †Excitation and emission maxima, in nm.

Before Starting

Materials Required but Not Provided

- 2–4 mg/mL protein sample in 50 mM Tris-HCl, pH 8.0 which has been labeled with an azide- or alkyne-modified biomolecule (Table 2). The sample can contain up to 1% SDS.
- Corresponding alkyne- or azide-containing detection reagent (Table 3)
- Dimethylsulfoxide (DMSO) or dimethylformamide (DMF)
- 18 megaOhm water
- Methanol
- Chloroform

Caution

The Copper (II) sulfate (Component B) is harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment. Avoid release to the environment. Refer to safety data sheets.

Preparing Stock Solutions

- 1.1 Solubilize the detection reagent selected from Table 3 in DMSO or DMF to make a 2–4 mM stock solution. After use, store any remaining stock solution at $\leq -20^{\circ}\text{C}$. When stored as directed, the stock solution is stable for up to 1 year.
- 1.2 Add the corresponding azide or alkyne-containing detection reagent (see Table 3), prepared in step 1.1, directly to the Click-iT[®] reaction buffer (Component A) for a final concentration of 40 μM . A total volume of 100 μL of this azide- or alkyne-containing reaction buffer is required for each click detection reaction. After use, store any remaining stock solution at $\leq -20^{\circ}\text{C}$. When stored as directed, the stock solution is stable for up to 1 year.
- 1.3 Add 500 μL of deionized water to the Click-iT[®] reaction buffer additive 1 (Component C) and mix until fully dissolved. After use, store any remaining stock solution at $\leq -20^{\circ}\text{C}$. When stored as directed, the stock solution is stable for up to 1 year.

Note: The additive is a reducing agent which can oxidize if not properly handled. Upon oxidation, the additive turns from colorless to brown. Discard solutions that are brown in color.

- 1.4 Add 540 μL of deionized water to the Click-iT[®] reaction buffer additive 2 (Component D) and mix until fully dissolved. After use, store any remaining stock solution at $\leq 6^\circ\text{C}$. When stored as directed, the stock solution is stable for up to 1 year.

Experimental Protocols

Performing the Click Reaction

- 2.1 Add the following to a 1.5 mL microcentrifuge tube:
 - Up to 200 μg in a maximum volume of 50 μL of azide- or alkyne-labeled protein in 50 mM Tris-HCl, pH 8.0, containing up to 1% SDS.
 - 100 μL of the Click-iT[®] reaction buffer prepared in step 1.2.
 - Sufficient volume of 18 megaOhm water for a final volume of 160 μL .
- 2.2 Cap the tube and vortex for 5 seconds.
- 2.3 Add 10 μL of CuSO_4 (Component B) and vortex for 5 seconds.
- 2.4 Add 10 μL of Click-iT[®] reaction buffer additive 1 solution (prepared in step 1.3) and vortex for 5 seconds. Wait for 2–3 minutes, but not longer than 5 minutes, before proceeding to step 2.6.
- 2.5 Add 20 μL of Click-iT[®] reaction buffer additive 2 solution (prepared in step 1.4) and vortex for 5 seconds. This solution turns bright orange.
- 2.6 Vortex continuously or rotate end-over-end for 20 minutes using a rotator. If using tetramethylrhodamine (TAMRA) as the detection reagent, **protect this solution from light**.

Preparing Sample for 1D or 2D-Analysis

Note: If you used the Click-iT[®] O-GlcNAc Enzymatic Labeling System (Cat. no. C33368), steps 3.1–3.8 are not recommended for the α -crystallin positive control.

- 3.1 Add 600 μL of methanol to the reaction mixture and vortex briefly.
- 3.2 Add 150 μL of chloroform and vortex briefly.
- 3.3 Add 400 μL of 18 megaOhm water and vortex briefly.
- 3.4 Centrifuge for 5 minutes at 13,000–18,000 $\times g$, then carefully remove and discard as much of the upper aqueous phase as possible while leaving the interface layer containing the protein precipitate intact.

Note: The upper phase is bright orange. The lower phase is pink if TAMRA is used or colorless if biotin is used.
- 3.5 Add 450 μL of methanol to the tube and vortex briefly.
- 3.6 Centrifuge for 5 minutes at 13,000–18,000 $\times g$ to pellet the protein. Discard the supernatant.
- 3.7 Add 450 μL of methanol to the tube and vortex briefly. Centrifuge and discard the supernatant. This second methanol wash step serves to remove residual reaction components.

- 3.8 Cover the tube with a lint-free tissue and keep the tube cap open. Allow the pellet to air-dry, for 15 minutes to overnight.
- 3.9 Cap the tube and store the sample at -20°C until use.

1D or 2D Analysis To verify the protein concentration, use the EZQ[®] Protein Quantitation Kit (Cat. no. R33200) as this kit is compatible with 1D and 2D sample loading buffers and requires only 1 μL of the sample.

- 4.1 Resolubilize the precipitated sample (from step 3.8) in 1D or 2D gel electrophoresis sample loading buffer. A target concentration of approximately 1–2 mg/mL is suggested.
- For 1D SDS gels, vortex the sample for 10 minutes followed by heating for 10 minutes at 70°C .
 - For 2D urea gels, vortex the sample for 10 minutes, if necessary for solubilization, heat for 10 minutes at 37°C .
- 4.2 Briefly, spin the protein sample before loading to remove any unsolubilized material. Recommended loading amounts for 1D minigels are 5–20 μg .
- 4.3 If using the Click-iT[®] O-GlcNAc Enzymatic Labeling System (Cat. no. C33368), add the sample loading buffer directly to the α -crystallin positive control. The α -crystallin concentration is $\sim 0.1 \mu\text{g}/\mu\text{L}$ before the addition of sample loading buffer. Load 0.1–1.0 μg (5–50 pmol) on the gel.
- 4.4 Perform electrophoresis as recommended by the manufacturer.
- 4.5 Following electrophoresis, remove the gel from the cassette.
- 4.6 Image Bis-Tris gels immediately after removal from the cassette or after a short 5 minute water wash. If imaged immediately, use water to rinse some of the SDS on the side of the gel that will be in contact with the imager tray. Add a little water to the imager tray before laying the gel down to prevent the gel from sticking.
- Wash the Tris-Glycine gels for 5 minutes with deionized water and image.
- 4.7 If the TAMRA detection reagent was used, image directly or perform a western transfer procedure. If biotin detection reagent was used, perform a western transfer procedure.
- 4.8 After imaging gels with TAMRA-labeled samples, the samples can be fixed and stained with SYPRO[®] Ruby total protein stain, Pro-Q[®] Emerald glycoprotein stain, or Pro-Q[®] Diamond phosphoprotein stain, or bands can be excised for subsequent mass spectrometry analysis.

References

1. ChemBioChem 4, 1147 (2003); 2. J Am Chem Soc 125, 3192 (2003); 3. Angew Chem Int Ed Engl 41, 2596 (2002); 4. Angew Chem Int Ed Engl 40, 2004 (2001); 5. J Am Chem Soc 130, 11576 (2008); 6. Proc Natl Acad Sci 103, 9482 (2006).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
C10276	Click-iT® Protein Reaction Buffer Kit	1 kit
Related Products		
B10184	biotin azide	0.5 mg
B10185	biotin alkyne	0.5 mg
C10186	Click-iT® HPG (L-homopropargylglycine) *for nascent protein synthesis*	5 mg
C10102	Click-iT® AHA (L-azidohomoalanine) *for nascent protein synthesis*	5 mg
C10248	Click-iT® farnesyl alcohol, azide *mixed isomers*	1 mg
C10249	Click-iT® geranylgeranyl alcohol, azide *mixed isomers*	1 mg
C10264	Click-iT® fucose alkyne (tetraacetyl fucose alkyne)	5 mg
C10265	Click-iT® palmitic acid, azide (15-azidopentadecanoic acid)	1 mg
C10268	Click-iT® myristic acid, azide (12-azidododecanoic)	1 mg
C33365	Click-iT® GalNAz metabolic glycoprotein labeling reagent (tetraacetylated <i>N</i> -azidoacetylgalactosamine) *for O-linked glycoproteins* *5.2 mg*	1 each
C33366	Click-iT® ManNAz metabolic glycoprotein labeling reagent (tetraacetylated <i>N</i> -azidoacetyl-D-mannosamine) *for sialic acid glycoproteins* *5.2 mg*	1 each
C33367	Click-iT® GlcNAz metabolic glycoprotein labeling reagent (tetraacetylated <i>N</i> -azidoacetylglucosamine) *for O-GlcNAc-modified proteins* *5.2 mg*	1 each
C33368	Click-iT® O-GlcNAc Enzymatic Labeling System	1 kit
T10182	tetramethylrhodamine (TAMRA) azide (tetramethylrhodamine 5-carboxamido-(6-azidohexanyl)) *5-isomer*	0.5 mg
T10183	tetramethylrhodamine (TAMRA) alkyne (5-carboxytetramethylrhodamine, propargylamide) *5-isomer*	0.5 mg
P21857	Pro-Q® Emerald 300 Glycoprotein Gel and Blot Stain Kit *10 minigels or minigel blots*	1 kit
P21875	Pro-Q® Emerald 488 Glycoprotein Gel and Blot Stain Kit *10 minigels or minigel blots*	1 kit
R33200	EZQ® Protein Quantitation Kit	1 kit
S12000	SYPRO® Ruby protein gel stain	1 L

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