Copper-less Click Chemistry Reagents

Table 1. Contents and storage information.

Material	Amount	Storage	Storage
DIBO containing click chemistry reagent	Varies (see product label)	• ≤–6°C • Desiccate • Protect from light	When stored as directed, the product is stable for 6 months
Approximate fluorescence excitation/emission maxima: See Table 2.			

Introduction

Click chemistry describes a class of chemical reactions that use bio-orthogonal or biologically unique moities to label and detect a molecule of interest in a two-step procedure. Classic click reaction involves a copper-catalyzed triazole formation from an azide and an alkyne.[1] The azide and alkyne moieties can be used interchangeably; either one can be used to tag the molecule of interest, while the other is used for subsequent detection. The azides and alkynes are biologically unique, inert, and stable.

One major shortcoming of the copper-catalyzed click reaction is the fact that Cu(II) as well as the Cu(I), which is produced in the presence of ascorbate, are highly cytotoxic.[2] A means of achieving click reactions while maintaining cell viability is the introduction of cyclooctynes, where the strain in the eight-membered ring allows the reaction with azides to occur in the absence of catalysts. One such class of reagents is comprised of the so-called DIBO compounds.[3] Enzymatically, chemically, or metabolically azide-modified macromolecules can now be labeled without the metal catalysts, which allows not only the study of live cells but also prevents damage of proteins, in particular fluorescent proteins like GFP.

The characteristics of copper-less click reactions include:

- Efficiency—the reaction between the detection moieties is complete in 1 hour and does not require extreme temperatures or solvents.
- Stability—the reaction product contains an irreversible, covalent bond.
- Biologically inert—the components of the reaction do not undergo any side reactions.
- Specificity—the reaction between the label and detection tag is selective and specific.
- Applicability to biological samples—the click chemistry-labeled molecules can be applied to complex biological samples and easily detected with high sensitivity and low background, as opposed to traditional chemical reactions that use succinimidyl esters or maleimides that target amines and sulfhydryls, which are not unique functional groups.
- Preservation of protein function and cell viability—the copper-less click chemistry reagents involving DIBO moieties are independent of millimolar concentrations of copper ions, which are cytotoxic and can impair the biological activity of proteins.

Several dye containing DIBO derivatives, as well as a biotin derivative and reactive probes containing DIBO compounds capable of modifying amine, cysteine and carbox groups are available from Invitrogen, complementing the wide variety of azide- or alkyne-containing dyes, haptens, and biomolecules for use in click reactions. A general protocol for the copper-less click reaction between an azide and a DIBO is described below.

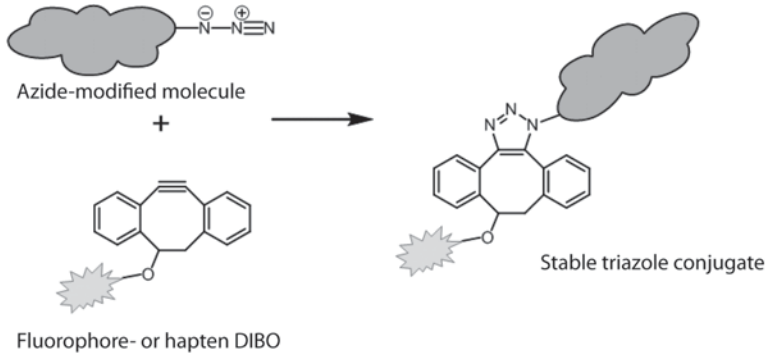

Figure 1. Copper-less click azide/DIBO reaction. The azide and alkyne moieties are interchangeable. The molecule can be labeled with a DIBO and reacted with a fluorophore or hapten-azide.

Table 2. DIBO modified fluorophores and haptens.

Label	Ex/Em*	Cat. no.	Use
Click-iT® DIBO-Alexa Fluor® 488	495/519	C10405	Fluorescent dye or hapten
Click-iT® DIBO-Alexa Fluor® 555	555/565	C10406	Fluorescent dye
Click-iT® DIBO-Alexa Fluor® 594	590/617	C10407	
Click-iT® DIBO-Alexa Fluor® 647	650/655	C10408	
Click-iT® DIBO TAMRA	555/580	C10410	
Click-iT® DIBO-biotin	NA	C10412	Hapten, Avidin binding
*Fluorescence excitation and emission maxima in nm. NA = not applicable.			

Table 3. DIBO compounds containing reactive moieties.

Compound	Cat. no.	Reactivity
Click-iT® DIBO-amine	C10411	Carboxylic acids
Click-iT® DIBO-maleimide	C10413	Thiols
Click-iT® DIBO-succinimidyl ester	C10414	Primary amines
*Fluorescence excitation and emission maxima in nm. NA = not applicable.		

Before You Begin

Molecules Key components to copper-less click reactions are an azide labeled molecule and a DIBO modified molecule

Solvent DIBO-derivatives modified with fluorophores, haptens, or reactive probes are hydrophobic molecules. We recommend that you dissolve these molecules in high quality, anhydrous dimethylsulfoxide (DMSO) or dimethylformamide (DMF). For live cell work, DMSO is the preferred co-solvent (final concentration < 0.5%).

Experimental Protocols

Live Cell Labeling

1.1 Grow mammalian cells (e.g., HeLa, U-2 OS) in an appropriate medium at 37°C in 5% CO_2.

1.2 Supplement the growth medium with an azide-derivatized metabolite. (e.g., Click-iT® ManNAz, Cat. no. C33366) and grow the cells for 2 to 3 days.

1.3 Wash the cells two times with D-PBS (Cat. no. 14190-144) containing 1% FBS (Cat. no. 16000).

1.4 Label the azide-modified macromolecules at room temperature in the dark for 1 hour with 5 to 30 µM of DIBO-Alexa Fluor® in D-PBS containing 1% FBS.

1.5 Wash the cells four times with D-PBS containing 1% FBS.

1.6 Fix the cells with 4% formaldehyde in D-PBS for 15 minutes at room temperature.

1.7 Wash the cells with D-PBS.

1.8 *Optional*: Counterstain the cells for 15 minutes at room temperature with Hoechst 33342 (Cat. no. H3570) in D-PBS.

1.9 Wash the cells two times with D-PBS.

1.10 Image the cells.

Note: You can image live cells after step 1.5 or after fixation without counterstaining (step 1.7). You can use multi-well plates as well as cover slips. If you are using cover slips, we recommend using the ProLong® Gold antifade reagent (Cat. no. P36930).

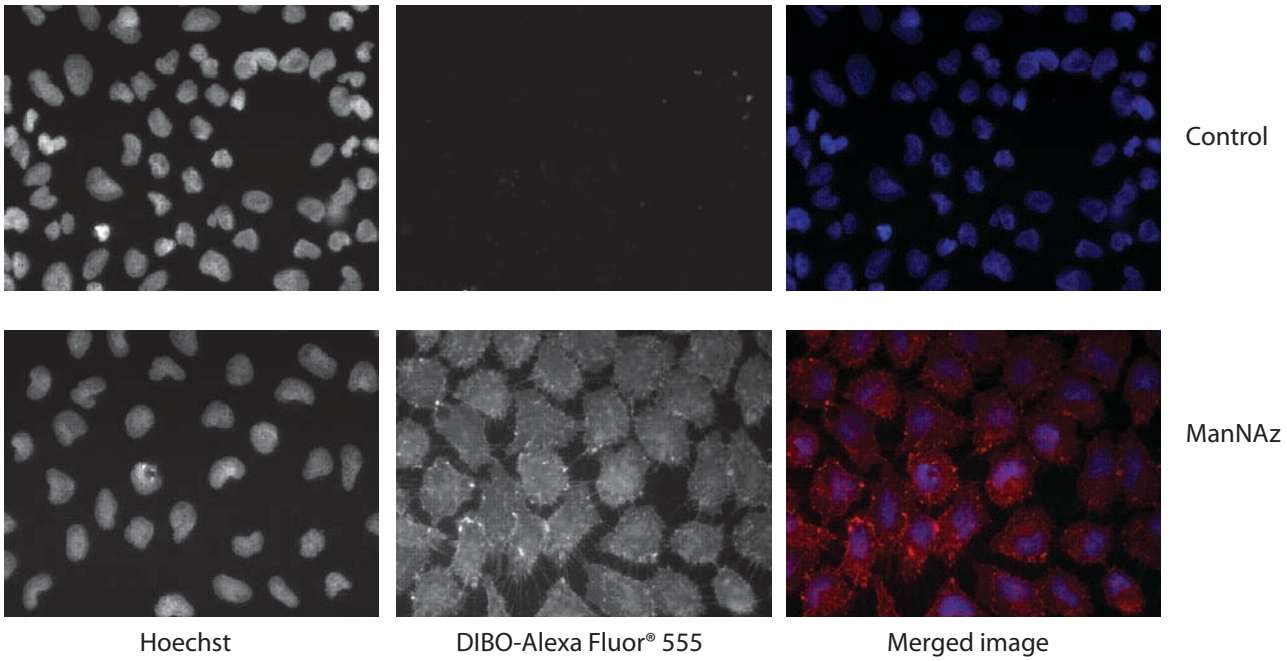

Figure 2. U-2 OS cells labeled live with Click-iT® DIBO-Alexa Fluor® 555 and imaged after fixation. U-2 OS cells were grown in a 12-well plate with cover slips in MEM supplemented with 10%FBS at 37°C and 5% CO_2. The cells were treated for three days with 50 µM tetraacetylated N-azidoacetyl-D-mannosamine (Click-iT® ManNAz, Cat. no. C33366) or an equal volume of solvent only (DMSO, control). After washing cells with D-PBS supplemented with 1%FBS, labeling with 30 µM Click-iT® DIBO-Alexa Fluor® 555 was carried out in the same buffer for one hour at room temperature. Following fixation with 4% formaldehyde and counterstaining with Hoechst 33342, the images were obtained with a Zeiss Axioscope at 40x magnification.

Protein Labeling

2.1 Introduce azide into proteins e.g. GalNAz in antibodies using the Click-iT® O-GlcNAc Enzymatic Labeling System (Cat. no. C33368).

2.2 Modify the protein-bound azide with a DIBO-modified fluorophore. Incubate the protein in TBS with 5 to 10 µM DIBO-label for at least 1 hour at room temperature.

2.3 Remove the excess label.

2.4 Analyze the modified protein.

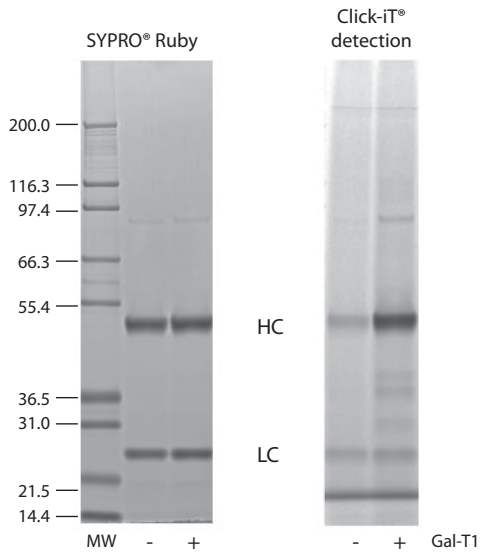

Figure 3. Labeling GalNAz modified antibody with Click-iT® DIBO-Alexa Fluor® 647. A Mouse monoclonal antibody against TSH was enzymatically modified with UDP-GalNAz using the mutant β1,4-galactosyltransferase β1,4Gal-T1-Y289L (+). In a control sample no enzyme was added to the reaction mix (-). 1 µg IgG in 14 µL TBS was incubated for one hour at room temperature with 5 µM Click-iT® DIBO-Alexa Fluor® 647 (C10408). 1 µL 1 M DTT was added followed by 5 µL 4X NuPAGE® LDS Sample Buffer. Heavy and light chains of the antibody were separated by 1D PAGE on NuPAGE® Novex® 4–12% Bis-Tris gels. Click-iT® DIBO-Alexa Fluor® 647 labeled gels were imaged on the FLA-9000 laser scanner (Fuji) using 635 nm excitation and an LPR emission filter (right panel). The gels were then fixed and post-stained with SYPRO® Ruby protein gel stain and imaged on the on the FLA-9000 laser scanner using 473 nm excitation and an LPG emission filter (left panel). MW contains a molecular weight standard (Mark12™ Unstained Standard, Cat. no. LC5677). HC and LC indicates the position of the heavy and light chain of the antibody, respectively.

References

1. Angew Chem Int Ed Engl 41, 2596 (2002); **2.** J Am Chem Soc 12511164 (2003); **3.** Angew Chem Int Ed Engl 47, 2253(2008).

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

Cat. no.	Product Name	Unit Size
C10405	Click-iT® DIBO-Alexa Fluor® 488 *for Cu-Free click chemistry*	0.5 mg
C10406	Click-iT® DIBO-Alexa Fluor® 555 *for Cu-Free click chemistry*	0.5 mg
C10407	Click-iT® DIBO-Alexa Fluor® 594 *for Cu-Free click chemistry*	0.5 mg
C10408	Click-iT® DIBO-Alexa Fluor® 647 *for Cu-Free click chemistry*	0.5 mg
C10410	Click-iT® DIBO TAMRA *for Cu-Free click chemistry*	0.5 mg
C10411	Click-iT® DIBO amine *for Cu-Free click chemistry*	1.0 mg
C10412	Click-iT® DIBO biotin *for Cu-Free click chemistry*	1.0 mg
C10413	Click-iT® DIBO maleimide *for Cu-Free click chemistry*	1.0 mg
C10414	Click-iT® DIBO succinimidyl ester *for Cu-Free click chemistry*	1.0 mg
Related Products		
A10044	EdU (5-ethynyl-2′-deoxyuridine)	50 mg
A10266	Alexa Fluor® 488 azide (Alexa Fluor® 488 5-carboxamido-(6-azidohexanyl), bis(triethylammonium salt))	0.5 mg
A10267	Alexa Fluor® 488 alkyne (Alexa Fluor® 488 5-carboxamido-(propargyl), bis(triethylammonium salt))	0.5 mg
A20012	Alexa Fluor® 555 azide, triethylammonium salt	0.5 mg
A20013	Alexa Fluor® 555 alkyne, triethylammonium salt	0.5 mg
A10270	Alexa Fluor® 594 azide (Alexa Fluor® 594 carboxamido-(6-azidohexanyl), bis(triethylammonium salt))	0.5 mg
A10275	Alexa Fluor® 594 alkyne (Alexa Fluor® 594 carboxamido-(5-(and 6-)propargyl), bis(triethylammonium salt))	0.5 mg
A10277	Alexa Fluor® 647 azide, triethylammonium salt	0.5 mg
A10278	Alexa Fluor® 647 alkyne, triethylammonium salt	0.5 mg
A10279	alkyne, succinimidyl ester (3-propargyloxypropanoic acid, succinimidyl ester)	1 mg
A10280	azido (PEO)4 propionic acid, succinimidyl ester (3-(azidotetra(ethyleneoxy))propionic acid, succinimidyl ester)	1 mg
B10184	biotin azide	1 mg
B10185	biotin alkyne	1 mg
C10102	Click-iT® AHA (L-azidohomoalanine) *for nascent protein synthesis*	5 mg
C10186	Click-iT® HPG (L-homopropargylglycine) *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 acid)	1 mg
C10269	Click-iT® Cell Reaction Buffer Kit.	1 kit
C10276	Click-iT® Protein Reaction Buffer Kit	1 kit
C33365	Click-iT® GalNAz metabolic glycoprotein labeling reagent (tetraacetylated N-azidoacetylgalactosamine) *for O-linked glycoproteins**5.2 mg*	1 each
C33366	Click-iT® ManNAz metabolic glycoprotein labeling reagent (tetraacetylated N-azidoacetyl-D-mannosamine) *for sialic acid glycoproteins* *5.2 mg*	1 each
C33367	Click-iT® GlcNAz metabolic glycoprotein labeling reagent (tetraacetylated N-azidoacetylglucosamine) *for O-GlcNAC-modified proteins* *5.2 mg*	1 each
C33368	Click-iT® O-GlcNAc Enzymatic Labeling System *for O-linked GlcNAc glycoproteins* *10 labelings*	1 kit
C33370	Click-iT® Tetramethylrhodamine (TAMRA) Protein Analysis Detection Kit *UV/532 nm excitation* *10 reactions*	1 kit
C33371	Click-iT® Dapoxyl® Protein Analysis Detection Kit *for UV excitation* *10 reactions*	1 kit
C33372	Click-iT® Biotin Protein Analysis Detection Kit *10 reactions*	1 kit
E10187	EdU (5-ethynyl-2′-deoxyuridine)	50 mg
I10188	iodoacetamide azide	1 mg
I10189	iodoacetamide alkyne	1 mg
O10180	Oregon Green® 488 azide (Oregon Green® 488 6-carboxamido-(6-azidohexanyl), triethylammonium salt)	0.5 mg
O10181	Oregon Green® 488 alkyne *6-isomer*	0.5 mg
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

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