

## Click Chemistry Reagents

**Table 1.** Contents and storage information.

Material	Amount	Storage	Stability
Alkyne or azide-containing click chemistry reagent	Varies, see product label	<ul style="list-style-type: none"> <li>• <math>\leq -20^{\circ}\text{C}</math></li> <li>• Desiccate</li> <li>• Protect from light</li> </ul>	When stored as directed the product is stable for 6–12 months.
<b>Approximate fluorescence excitation/emission maxima:</b> See Table 2.			

### Introduction

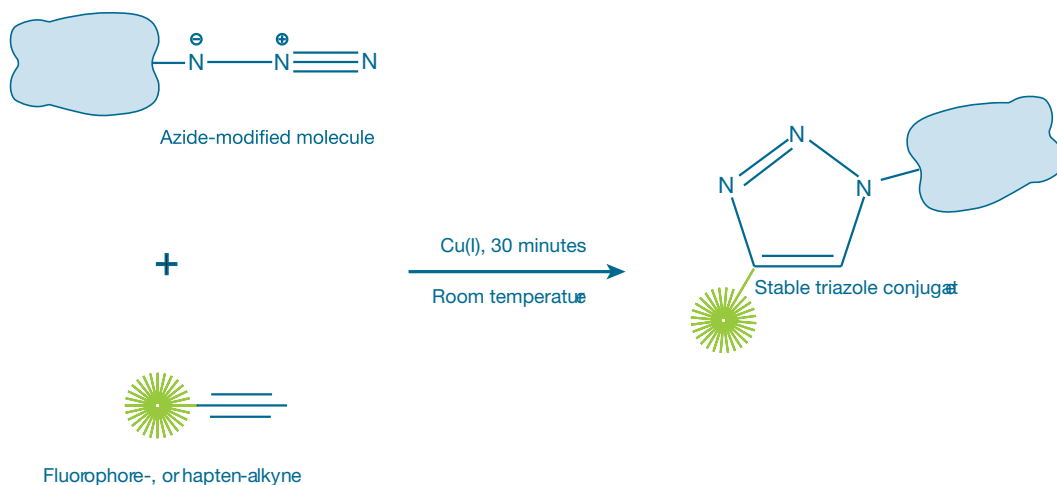
Click chemistry describes a class of chemical reactions that use bio-orthogonal or biologically unique moieties to label and detect a molecule of interest using a two-step procedure.<sup>1–4</sup> The two-step click reaction involves a copper-catalyzed triazole formation from an azide and an alkyne (Figure 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, stable, and extremely small (Figure 2).

Click chemistry can be used when methods such as direct labeling or the use of antibodies are not applicable or efficient. The click chemistry label is small enough that tagged molecules (e.g., nucleotides<sup>5</sup>, sugars<sup>6</sup>, and amino acids<sup>7</sup>) are acceptable substrates for the enzymes that assemble these building blocks into biopolymers. The small size of click detection molecules allows them to easily penetrate complex samples, including intact, supercoiled DNA, with only mild permeabilization required.

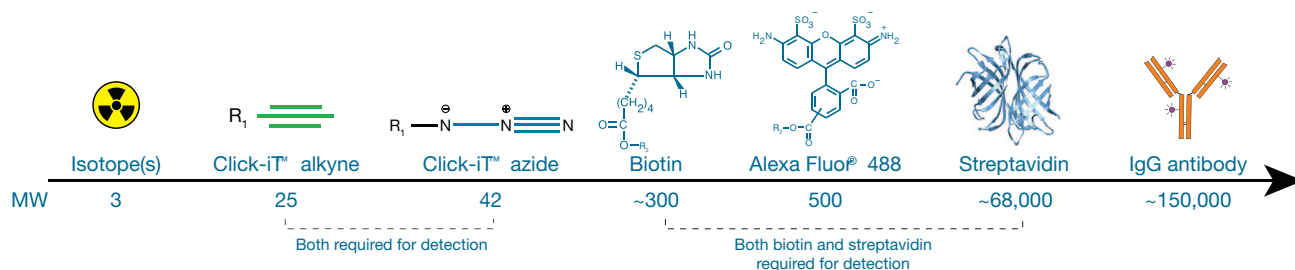
The characteristics of click reactions include:

- **Efficiency**—the reaction between the detection moieties is complete in less than 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, unlike traditional chemical reactions that use succinimidyl esters or maleimides that target amines and sulfhydryls, which are not unique functional groups.

A wide variety of azide- or alkyne-containing dyes, haptens, and biomolecules for use in click reactions are available from Invitrogen (Tables 2–4). A general protocol for the copper-catalyzed click reaction between an azide and an alkyne is described below.



**Figure 1.** Click azide/alkyne reaction. The azide and alkyne moieties are interchangeable, whereupon the molecule can be labeled with an alkyne and reacted with a fluorophore or haptene-azide.



**Figure 2.** Relative size of detection molecules commonly used in cellular analysis.

**Table 2.** Azide- or alkyne-modified fluorophores and haptens.

Label	Ex/Em*	Azide or alkyne	Cat. no.	Use
Alexa Fluor <sup>®</sup> 488	495/519	azide	A10266	Fluorescent dye or hapten
		alkyne	A10267	
Alexa Fluor <sup>®</sup> 555	555/565	azide	A20012	Fluorescent dye
		alkyne	A20013	
Alexa Fluor <sup>®</sup> 594	590/617	azide	A10270	Fluorescent dye
		alkyne	A10275	
Alexa Fluor <sup>®</sup> 647	650/655	azide	A10277	Fluorescent dye
		alkyne	A10278	
Biotin	Not applicable	azide	B10184	Hapten
		alkyne	B10185	
Oregon Green <sup>®</sup> 488	496/524	azide	O10180	Fluorescent dye or hapten
		alkyne	O10181	
Tetramethylrhodamine	555/580	azide	T10182	Fluorescent dye or hapten
		alkyne	T10183	

\*Fluorescence excitation and emission maxima in nm.

**Table 3.** Azide- or alkyne-modified biomolecules.

Compound	Cat. no.	Azide or alkyne	Application
Edu (5'-ethynyl-2'-deoxyuridine)	A10044, E10187	alkyne	For nascent DNA synthesis
Click-iT® AHA (L-azidohomoalanine)	C10102	azide	Nascent protein synthesis
Click-iT® HPG (L-homopropargylglycine)	C10186	alkyne	
Click-iT® farnesyl alcohol, azide	C10248	azide	Farnesylated proteins
Click-iT® geranylgeranyl alcohol, azide	C10249	azide	Geranylgeranylated proteins
Click-iT® fucose alkyne (tetraacetylfucose alkyne)	C10264	alkyne	Fucosylated glycans
Click-iT® palmitic acid, azide	C10265	azide	Palmitoylated proteins
Click-iT® myristic acid, azide	C10268	azide	Myristoylated proteins
Click-iT® GalNAz (tetraacetylated N-azidoacetylgalactosamine)	C33365	azide	O-Linked glycoproteins
Click-iT® ManNAz (tetraacetylated N-azidoacetyl-D-mannosamine)	C33366	azide	Sialic acid-modified glycoproteins
Click-iT® GlcNAz (tetraacetylated N-azidoacetylglucosamine)	C33367	azide	O-GlcNAz-modified glycoproteins

**Table 4.** Reactive azides and alkynes.

Reactivity	Reactive moiety	Azide or alkyne	Cat. no.
Primary amines	Succinimidyl ester	azide	A10280
		alkyne	A10279
Thiols	Iodoacetamide	azide	I10188
		alkyne	I10189

## Before Starting

---

### Azide- and Alkyne-modified-Molecules

The key components to any click reaction are an azide labeled molecule and an alkyne labeled molecule.

### Solvent

- Most alkyne- and azide-modified fluorophores, haptens, and reactive probes are hydrophobic molecules. We recommend that you dissolve these molecules in high-quality, anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO).
- For azide- and alkyne-modified biomolecules (Table 3), refer to the specific protocol supplied with the products.

## Click Reaction Conditions

---

### Use of Copper as a Catalyst

In addition to the azide and alkyne labeled molecules, copper (I) is required to catalyze the reaction. We recommend using copper (II) sulfate in the presence of a reductant such as ascorbic acid to generate copper (I). The use of copper (I) directly is less favored due to the ease with which it is oxidized to the non-catalytic copper (II) species. The preferred method is the reduction of copper (II) sulfate *in situ* to obtain copper (I).

### pH and Temperature

The click reaction is highly efficient and extremely tolerant of a wide variety of conditions. The click reaction occurs at pH values ranging from 3 to 12, at room temperature, generally in less than one hour. Reaction rates have been found to increase slightly at lower pH levels where copper (I) is more soluble, and therefore more readily available for catalysis. Although the reaction is not light sensitive, protect the reaction from light for light-sensitive fluorophores.

### Click-iT® Reaction Buffer Kits

For convenience, Invitrogen offers Click-iT® Reaction Buffer Kits for protein or cell samples labeled with an azide- or alkyne-tagged biomolecule. The Click-iT® Cell Reaction Buffer Kit (Cat. no. C10269) includes sufficient reagents to perform 50 reactions based on a 0.5 mL reaction volume for subsequent analyses by flow cytometry, fluorescence microscopy, or high content screening (HCS). The Click-iT® Protein Reaction Buffer Kit (Cat. no. C10276) includes everything required to perform the click reaction of proteins for subsequent standard protein biochemical analyses (e.g., western blots, mass spectrometry).

## 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. Proc Natl Acad Sci 105, 2415 (2008); 6. J Am Chem Soc 130, 11576 (2008); 7. 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
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) <sub>4</sub> propionic acid, succinimidyl ester (3-(azidotetra(ethyleneoxy))propionic acid, succinimidyl ester)	1 mg
B10184	biotin azide	1 mg
B10185	biotin alkyne	1 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
<b>Related Products</b>		
A10044	EdU (5-ethynyl-2'-deoxyuridine)	50 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 <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- <i>D</i> -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 *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)	500 mg

## Contact Information

---

### Molecular Probes, Inc.

29851 Willow Creek Road  
Eugene, OR 97402  
Phone: (541) 465-8300  
Fax: (541) 335-0504

### Customer Service:

6:00 am to 4:30 pm (Pacific Time)  
Phone: (541) 335-0338  
Fax: (541) 335-0305  
probesorder@invitrogen.com

### Toll-Free Ordering for USA:

Order Phone: (800) 438-2209  
Order Fax: (800) 438-0228

### Technical Service:

8:00 am to 4:00 pm (Pacific Time)  
Phone: (541) 335-0353  
Toll-Free (800) 438-2209  
Fax: (541) 335-0238  
probestech@invitrogen.com

### Invitrogen European Headquarters

Invitrogen, Ltd.  
3 Fountain Drive  
Inchinnan Business Park  
Paisley PA4 9RF, UK  
Phone: +44 (0) 141 814 6100  
Fax: +44 (0) 141 814 6260  
Email: euroinfo@invitrogen.com  
Technical Services: eurotech@invitrogen.com

**For country-specific contact information,  
visit [www.invitrogen.com](http://www.invitrogen.com).**

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

### Limited Use Label License No. 223: Labeling and Detection Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2009, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.