Click-iT® Cell Reaction Buffer Kit

Catalog no. C10269

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Click-iT® cell reaction buffer (Component A)	4 mL	10X solution containing Tris-buffered saline	• 2–6˚C • Desiccate	When stored as directed this kit is stable for 1 year.
Copper (II) sulfate ($CuSO_4$, Component B)	550 µL	100 mM aqueous solution		
Click-iT® cell buffer additive (Component C)	80 mg	Not applicable		
Number of assays: Sufficient material is supplied for 50 reactions based on a 0.5 mL reaction volume.				

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.[1-4] The 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. Click chemistry fills the void when methods such as direct labeling with a fluorophore or biotin, or the use of antibodies are not applicable or efficient. Azides and alkynes are small enough that tagged molecules (e.g., nucleotides[5], sugars[6], and amino acids[7]) are acceptable substrates for the enzymes that assemble these building blocks into biopolymers and the detection molecules can easily penetrate complex samples, including intact supercoiled DNA, with only mild permeabilization required.

The Click-iT® Cell Reaction Buffer Kit allows you to easily perform the click reaction with cells labeled with an azide or alkyne and the corresponding click detection reagent. Prior to or directly after the click reaction, cells can be stained with additional detection reagents for deeper biological insight (Table 2). The Click-iT® Cell Reaction Buffer Kit includes sufficient reagents to perform 50 reactions based on a 0.5 mL reaction volume for subsequent analysis by flow cytometry, fluorescence microscopy, or high content screening (HCS).

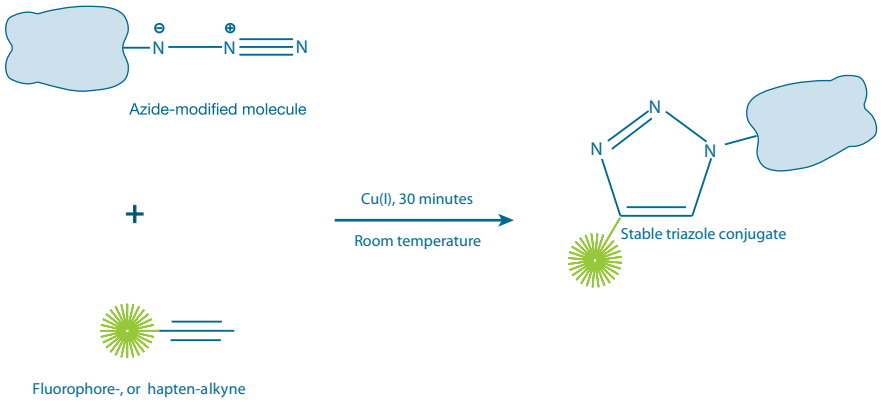

Figure 1. Click azide/alkyne reaction. The azide and alkyne moieties are interchangeable, whereupon the molecule can be labeled with an alkyne and react with a fluorophore or hapten-azide.

Table 2. Detection reagent compatibility with click chemistry.

Detection Reagent	Compatibility*
Qdot® nanocrystals	Use Qdot® nanocrystals ***after*** the Click-iT® detection reaction.
Fluorescent proteins (i.e., GFP)	Use organic dye-based reagents such as TC-FlAsH™ or TC-ReAsH™ reagents, for protein expression or anti-GFP rabbit or chicken antibodies ***after*** the Click-iT® detection reaction.
Organic dyes such as Alexa Fluor® dyes, fluorescein (FITC)	Completely compatible with the Click-iT® detection reaction.
TC-FlAsH™ or TC-ReAsH™ reagents	Detect the tetracysteine (TC) tag with FlAsH™ or ReAsH™ reagents ***before*** the Click-iT® detection reaction.
Phalloidin	Detect the cytoskeleton with an antibody, i.e. anti-α-tubulin antibody.
*Compatibility indicates whether the detection molecule itself or detection method involve components that are unstable in the presence of the copper catalyst required for the Click-iT® detection reaction.	

Before You Begin

Materials Required but Not Provided

- Azide-labeled and alkyne-labeled molecules
- 1–3% Bovine serum albumin (BSA) in phosphate buffered saline (PBS), pH 7.1–7.4
- Fixative (e.g. 4% paraformaldehyde in PBS)
- Permeabilization reagent (e.g., 0.25% Triton® X-100 in PBS or 1% BSA with 0.1% saponin in PBS, preferred for flow assay)
- Deionized water or 18 megaohm purified water
- Flow tubes (for flow assay)
- Coverslips (for imaging assay)
- 96-well plate (for HCS assay)

Preparing 1X Click-iT® Cell Reaction Buffer

To make 40 mL 1X Click-iT® cell reaction buffer (Component A), transfer 4 mL solution from the Component A bottle to 36 mL deionized water. To make smaller amounts of 1X Click-iT® cell reaction buffer, dilute volumes from the Component A bottle 1:10 with deionized water. After use, store any remaining 1X Click-iT® cell reaction buffer at 2–6˚C. When stored as directed, the 1X Click-iT® cell reaction buffer is stable for 6 months.

Preparing Click-iT® Cell Buffer Additive

Add 4 mL deionized water to Click-iT® cell buffer additive (Component C), and mix until fully dissolved. After use, store any remaining Click-iT® cell buffer additive solution at ≤–20ºC. When stored as directed, the Click-iT® cell buffer additive solution is stable for up to 1 year. 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.

Experimental Protocol

1.1 Fix and permeabilize cells following a standard protocol.

1.2 Wash cells once with 1–3% BSA in PBS.

1.3 Prepare the Click-iT® reaction cocktail according to Table 3.

Note: Use the Click-iT® reaction cocktail within 15 minutes of preparation.

1.4 Add 0.5 mL Click-iT® reaction cocktail (prepared in step 1.3) to each sample. If adding to a cell pellet in a flow tube, mix well.

1.5 Incubate samples for 30 minutes at room temperature, and if using a fluorescent detection reagent, **protect from light**.

1.6 Wash cells once with 1–3% BSA in PBS.

1.7 Stain with a desired counter stain or antibodies prior to imaging or flow analysis.

Table 3. Click-iT® cell reaction cocktails.

Reaction Components	Number of Reactions						
	1	2	5	10	15	30	50
1X Click-iT® cell reaction buffer (prepared in **Before You Begin**)	440 µL	880 µL	2.2 mL	4.4 mL	6.6 mL	13.2 mL	22 mL
$CuSO_4$	10 µL	20 µL	50 µL	100 µL	150 µL	300 µL	500 µL
Click-iT® cell buffer additive (prepared in **Before You Begin**)	50 µL	100 µL	250 µL	500 µL	750 µL	1.5 mL	2.5 mL
alkyne or azide-modified molecule	Add to 1–5 µM final concentration*						
Total Volume	500 µL	1 mL	2.5 mL	5 mL	7.5 mL	15 mL	25 mL

*This final concentration is a starting point. Depending on the actual detection reagent used, the optimal final concentration may be lower or higher.

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
C10269	Click-iT® Cell Reaction Buffer Kit	1 kit
Related Products		
A10266	Alexa Fluor® 488 azide (Alexa Fluor® 488 5-carboxamido-(6-azidohexanyl), bis(triethylammonium salt)) *5-isomer*	0.5 mg
A10267	Alexa Fluor® 488 5-carboxamido-(propargyl), bis(triethylammonium salt)	0.5 mg
A10270	Alexa Fluor® 594 azide (Alexa Fluor® 594 carboxamido-(6-azidohexanyl) , triethylammonium salt) *mixed isomers*	0.5 mg
A10275	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
B10184	biotin azide	1 mg
B10185	biotin alkyne	1 mg
O10180	Oregon Green® 488 azide (Oregon Green® 6-carboxamido-(6-azidohexanyl) , triethylammonium salt) *6-isomer*	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
C10202	Click-iT® AHA (L-azidohomoalanine) *for nascent protein synthesis*	5 mg
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
A10044	EdU (5-ethynyl-2′-deoxyuridine)	50 mg
E10187	EdU (5-ethynyl-2′-deoxyuridine)	500 mg

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