USER GUIDE

Click-&-Go® Click Chemistry Reaction Buffer Kit

vector LABORATORIES

Together we breakthrough™

Cat. No. CCT-1001

Introduction

Click-&-Go® Click Chemistry Reaction Buffer Kit is general purpose click chemistry kit that provides all the necessary reagents to perform copper-catalyzed click reaction between any azide- and alkyne-tagged biomolecule in aqueous media. The kit includes specially formulated components to both catalyze and protect proteins during the click labeling reaction. Sufficient reagents are provided for 25 labeling reactions containing 50 µL protein solution (1-5 mg/mL).

Kit Contents

Component	Concentration	Amount
Reaction Buffer (Component A)	1X	3.0 mL
Copper (II) Sulfate (Component B)	20 mM	0.55 mL
Reducing Agent (Component C)		40 mg
Additive #1 (Component D)		20 mg

Materials Required but Not Provided

- Azide or alkyne detection reagents
- High-speed microcentrifuge
- 1.5 mL microfuge tubes
- 1-5 mg/mL azide or alkyne-labeled protein samples
- Solvents: methanol, chloroform, water

Additional information

- Final concentrations of an azide or alkyne detection reagent may range from 2 µM to 20 µM. Final concentrations below or above this range are also possible, and should be optimized per the specific application. We recommend starting with a final concentration of 20 µM, and titrating this amount down in case of high background.
- Compatible with protein extraction buffers containing 1% SDS, sodium phosphate buffers containing 1% NP-40, or RIPA Buffer (50 mM Tris-HCl, 150 mM sodium chloride).
- Caution- copper (II) sulfate solution is harmful to aquatic organisms and can cause damage to aquatic environments. Avoid release into the environment. Refer to SDS.

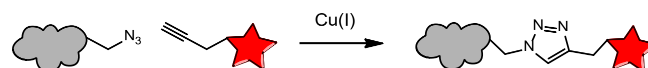

Figure 1. *Schematic representation of tagging of an azide-modified protein with an alkyne-detection reagent via Cu(I)-catalyzed click reaction.*

Material Preparation

Azide or Alkyne Detection Reagents	Prepare a stock solution of alkyne- or azide-functionalized detection reagents in appropriate water-miscible solvent or water.
Reaction Buffer (Component A)	Add a stock solution of azide or alkyne detection reagents directly to **Reaction Buffer (Component A)** for a final concentration of 40 µM. Store unused stock at -20°C. Stable for 1 year when stored as directed.
Copper (II) Sulfate (Component B)	Ready to use. Stable for 1 year when stored at ambient temperature
Reducing Agent (Component C)	Dissolve **Reducing Agent** in 500 µL of deionized water, vortex vigorously for 2 minutes or until pellet is completely dissolved. Store unused stock at -20°C. Stable for 1 year when stored as directed. *Note-* reducing agent is susceptible to oxidation and turns brown when oxidized. If solution appears brown do not use.
Additive 1 (Component D)	Dissolve **Additive 1** in 0.56 mL of deionized water, vortex until completely dissolved. Store unused stock at -20°C. Stable for 1 year when stored as directed.

Click Labeling Reaction

1. For each azide or alkyne- modified protein lysate sample, add the following to a 1.5 mL microfuge tube, then vortex briefly to mix.
   - 50 µL protein lysate (1-5 mg/mL) in in 50 mM Tris-HCl, pH 8.0, containing up to 1% SDS.
   - 110 µL Reaction Buffer (Component A)
2. Add 20 µL **Additive 1** (Component D), vortex briefly to mix.

©2023 Vector Laboratories. All rights reserved.

For research use only. Not intended for animal or human therapeutic or diagnostic use.

6737 Mowry Avenue, Newark CA 94560

customerservice@vectorlabs.com

vectorlabs.com

USER GUIDE

Click-&-Go® Click Chemistry Reaction Buffer Kit

vector LABORATORIES

Together we breakthrough™

3. Add 20 µL **Copper (II) Sulfate** (Component B), vortex briefly to mix.
4. Add 10 µL **Reducing Agent** (Component C), to initiate click reaction, vortex briefly to mix.
5. Protect reaction from light and allow click reaction to incubate for 30 minutes at room temperature.
6. Proteins in lysate are now click labeled and ready for downstream processing and/or analysis.

Preparation of Samples for Gel Analysis

1. Add 600 µL methanol to 200 µL reaction mixture, vortex briefly.
2. Add 150 µL chloroform, vortex briefly.
3. Add 400 µL dH_2O, vortex briefly.
4. Centrifuge for 5 minutes at 13,000-20,000 g, carefully remove upper aqueous layer without disturbing interface layer containing proteins. Note-upper aqueous layer may either be colorless or contain color depending on detection reagent.
5. Add 450 µL methanol, vortex briefly.
6. Centrifuge for 5 minutes at 13,000-20,000 g to pellet protein. Carefully, remove and discard supernatant.
7. Add 450 µL methanol, vortex briefly. Repeat step 6.
8. Open the lid to microfuge tube and allow protein pellet to air-dry for at least 15 minutes.
9. Cap and store labeled sample at -20°C until ready for use.

Gel Analysis

1. Add 50 µL gel electrophoresis sample buffer to protein pellet and vortex for 10 minutes to resuspend. Protein concentration will generally range from 1-5 µg/µL depending on starting protein concentration.
2. Briefly spin protein/sample buffer mix before loading to remove any material that may not have been completely resuspended. Recommended loading amount for 1 -dimensional mini-gels is 5-20 µg.
3. For SDS-PAGE gels, heat sample 10 minutes at 70°C prior to loading. For 2-dimensional gels resuspend in sample buffer, vortex for 10 minutes. Heat at 37°C for 10 minutes, if necessary.
4. Perform electrophoresis as directed by manufacturer.
5. Scan and image the gel immediately after removing gel from cassette.

Click-&-Go® is registered trademark of Vector Laboratories, Inc.
©2023 Vector Laboratories. All rights reserved.
For research use only. Not intended for animal or human therapeutic or diagnostic use.

6737 Mowry Avenue, Newark CA 94560
customerservice@vectorlabs.com
vectorlabs.com