USER GUIDE

Click-&-Go® Cell Reaction Buffer Kit

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Cat. No. CCT-1263

Introduction

The Click-&-Go® Protein Buffer Kit provides researchers everything required to perform the click reaction on cells tagged with an azide or alkyne and with the corresponding click detection reagent for subsequent downstream analysis by flow cytometry, fluorescence microscopy, or high content screening (HCS). Staining with additional detection reagents can performed prior or after the click reaction. Each kit includes sufficient material to perform 50 reactions based on 0.5 mL volume.

Kit Contents

Component	Concentration	Amount	Storage	Stability
Reaction Buffer (Component A)	10X	4.0 mL	2-8°C	Stable for at least 12 months when stored as directed.
Copper (II) Sulfate (Component B)	100 mM	600 µL		
Reducing Agent (Component C)		100 mg		

Materials Required but Not Provided

- Alkyne or azide-tagged cells
- Azide or alkyne detection reagents
- High-speed microcentrifuge
- Flow tubes (for flow assay)
- Coverslips (for imaging assay)
- 1.5 mL microfuge tubes
- 1-5 mg/mL azide or alkyne-labeled protein samples
- 18 mega-Ohm water
- Fixative (e.g. 4% paraformaldehyde in PBS)
- 96-well plate (for HCS assay)
- 1–3% Bovine serum albumin (BSA) in phosphate buffered saline (PBS), pH 7.1–7.4
- Permeabilization reagent (e.g., 0.25% Triton® X-100 in PBS or 1% BSA with 0.1% saponin in PBS, preferred for flow assay)

Additional information

- Final concentrations of an azide or alkyne detection reagent may range from 1 µM to 5 µM, depending on the actual detection reagent used. 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

  2 µM, and titrating this amount down in case of high background.
- Caution- copper (II) sulfate solution is harmful to aquatic organisms and can cause damage to aquatic environments. Avoid release into the environment. Refer to MSDS.

Material Preparation

Prepare Azide or Alkyne Detection Reagent	Prepare detection reagent of choice in DMSO to a concentration of 2.5-5 mM. After use, store unused detection reagent stock at -20°C for up to 1 year.
Reaction Buffer (Component A)	Prepare required amount of **1x Reaction Buffer** by dilution 10 fold with deionized water. For example, to make 5 mL of 1x Reaction Buffer transfer 0.5 mL directly from **10x Reaction Buffer (Component A)** into 4.5 mL of deionized water. Store unused **10x Reaction Buffer (Component A)** for up to 6 months.
Copper (II) Sulfate (Component B)	Ready to use. Stable for 1 year when stored at ambient temperature
Reducing Agent (Component C)	Dissolve **Reducing Agent (Component C)** in 4 mL of deionized water, vortex vigorously for 2 minutes or until pellet is completely dissolved. Store unused stock refrigerated 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.

Click Labeling Reaction

1. Fix and permeabilize cells following a standard protocol.
2. Wash cells once with 1-3% BSA in PBS.
3. Prepare the reaction cocktail according to the table below:

   Note: the reaction cocktail should be used within 10 minutes of preparation.

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Component	Number of Reactions						
	1	2	5	10	15	30	50
1x Reaction Buffer (Component A)	440 µL	880 µL	2.2 mL	4.4 mL	6.6 mL	13.2 mL	22 mL
Copper (II) Sulfate (Component B)	10 µL	20 µL	50 µL	100 µL	150 µL	300 µL	500 µL
Reducing Agent (Component C)	50 µL	100 µL	250 µL	500 µL	750 µL	1.5 µL	2.5 µL
Detection Reagent	1 to 5 µM final concentration, depending on the actual detection reagent used.						
Total Volume	500 µL	1 mL	2.5 mL	5 mL	7.5 mL	15 mL	25 mL

4. Add 0.5 mL reaction cocktail to each sample, mix well.
5. Protect reaction from light and vortex continuously or rotate end-over-end for 25-30 minutes at room temperature.
6. Wash cells once with 1–3% BSA in PBS.
7. Stain with a desired counter stain or antibodies prior to imaging or flow analysis.

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