

# Click-&-Go<sup>®</sup> Plus 647 Imaging Kit

**Cat. No.** CCT-1320

## Introduction

Click-&-Go<sup>®</sup> Plus 647 Imaging Kit is an all-inclusive kit optimized for imaging alkyne-tagged biopolymers with far-red-fluorescent, copper-chelating azide. Unlike picolyl azides, our copper-chelating azide reagents (azide plus reagents) incorporate a complete copper-chelating system in their structure and form strong, active copper complexes that react almost instantaneously with alkynes under diluted conditions. The kit provides far-red-fluorescent AFDye 647 Azide Plus and all the necessary reagents to perform at least 30 cell or tissue assays based on a total reaction volume of 500  $\mu$ L.

## Kit Contents

Component	Concentration	Amount
AFDye 647 Azide Plus (Component A)	n/a	1 vial
Reaction Buffer (Component B)	10x solution	4 mL
Copper Catalyst (Component C)	100x solution	0.5 mL
Reducing Agent (Component D)	n/a	400 mg
Wash Buffer (Component E)	n/a	25 mL

## Materials Required but Not Provided

- Alkyne-modified sample
- DMSO, deionized water ( $\text{dH}_2\text{O}$ )
- 1.5 mL microfuge tubes
- Coverslips/microscope slides, mounting media (for imaging)

### For cultured cells or tissue processing

- Fixative (e.g., 3.7% Formaldehyde in PBS)
- Wash buffer such as PBS, HBSS, or TBS (pH 7.2–7.6)
- Blocking agent such as 1–5% Bovine serum albumin (Fraction V, defatted BSA) in PBS, pH 7.4, or 5–10% animal serum in PBS, pH 7.4
- Optional: Permeabilization reagent (e.g., 0.5% Triton<sup>®</sup> X-100 in PBS, saponin)

**Note:** Permeabilization reagent is not required for surface labeling or labeling of lipid components

- Optional: Labeling reagents such as antibodies, avidin/streptavidin, or stains, as well as suitable diluents
- Optional: Mounting medium (for imaging)

## Additional information

- Final concentrations of an azide plus detection reagent may range from 0.25  $\mu$ M to 5  $\mu$ M. 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 5  $\mu$ M, and titrating this amount down in case of high background.
- Final reaction volumes may be scaled up or down. The protocol provides an example of a single click reaction with a total reaction volume of 500  $\mu$ L that would be suitable for a monolayer of adherent cells on an 18  $\times$  18-mm coverslip or for 100  $\mu$ L of suspension cells at a cell density of  $10^7$  cells/mL.
- For any cellular or non-cellular processing during the click reaction and after the attachment of the dye-azide, avoid extremes of pH, high salt concentrations, strong oxidizing or reducing agents, heavy metals, and quenching agents.
- 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.
- Wash Buffer (Component F) contains 2 mM sodium azide. Sodium azide is harmful to aquatic organisms and can cause damage to aquatic environments. Avoid release into the environment. Refer to MSDS.

## Fix and Permeabilize Cells

This protocol below provides general guidelines for fixation using 3.7% formaldehyde in PBS, followed by permeabilization with 0.5% Triton<sup>®</sup> X-100 reagent. However, other fixation/permeabilization protocols with reagents such as methanol and saponin can also be used.

- Optional: If desired, treat unfixed sample with antibodies against cell surface antigens.

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- Remove media from the sample and rinse it once with PBS. Then, add appropriate amount of 3.7% formaldehyde in PBS. Incubate for 15 minutes at room temperature.
- Remove the fixative and wash sample twice with PBS.
- Remove the wash solution and add an appropriate amount of 0.5% Triton<sup>®</sup> X-100 in PBS and incubate for 15 minutes at room temperature.

## Material Preparation

<b>AFDye 647 Azide Plus (Component A)</b>	Add 400 µL of deionized water or DMSO. Protect from light. Store at 4°C. This stock solution is stable for up to 6 months.
<b>Reaction Buffer (Component B)</b>	To prepare a required amount of <b>1x reaction buffer</b> , dilute the appropriate volume from <b>Reaction Buffer (Component B)</b> bottle 1:10 with deionized water. Store undiluted 10X reaction buffer at 2-8°C. The 10X solution is stable for 1 year.
<b>Copper Catalysts (Component C)</b>	Ready to use. When stored as directed, this stock solution is stable for up to 1 year.
<b>Reducing Agent (Component D)</b>	Prepare <b>1x solution of Reducing Agent (Component D)</b> that is enough for one day. Weigh out 20 mg of <b>Reducing Agent (Component D)</b> into 2 mL vial, add 1.8 mL of deionized water. Vortex until completely dissolved.  <i>Note-</i> reducing agent is susceptible to oxidation and turns brown when oxidized. We recommend always using freshly prepared solution of reducing agent.
<b>Wash Buffer (Component E)</b>	Ready to use. When stored as directed, this stock solution is stable for up to 1 year.

## Click Labeling Reaction

- Prepare required amount of **1x solution of Reducing Agent (Component D)**. This solution should be used on the same day.
- For labeling of fixed and permeabilized, cells remove the permeabilization buffer and wash the sample twice with PBS. Remove the wash solution.
- For each labeling reaction prepare a reaction cocktail in a 1.5 mL microfuge tube **in the order given**, and then vortex briefly to mix.

Component	Amount
1x Reaction Buffer (prepared in <b>Material Preparation</b> )	435 µL
AFDye 647 Azide Plus (prepared in <b>Material Preparation</b> )	10 µL
Copper Catalyst (Component C)	5 µL
1x Solution of Reducing Agent (prepared in <b>Step 1</b> )	50 µL

- Immediately** add the reaction cocktail to the sample. Evenly distribute the reaction cocktail over the sample.
- Protect reaction from light and allow click reaction to incubate for 20-30 minutes at room temperature.
- Remove the reaction cocktail and wash sample once with Wash Buffer (Component E).
- Remove the Wash Buffer and wash the sample once with PBS.
- Optional: If additional immunostaining is desired, incubate the sample with 3% BSA in PBS for 30-60 minutes to block non-specific interactions and proceed with antibody staining according to manufacture recommended protocol, protected from light.
- If additional staining is desired, proceed with fixed-cell stains (e.g., nuclear counterstain) following manufacturer's recommendations.
- The sample is now ready for downstream processing and/or analysis.