USER GUIDE

Click-&-Go® Plus 532 Imaging Kit

Together we breakthrough™

Cat. No. **CCT-1315**

Introduction

Click-&-Go® Plus 532 Imaging Kit is an all-inclusive kit optimized for imaging alkyne-tagged biopolymers with yellow-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 yellow-fluorescent AFDye 532 Azide Plus and all the necessary reagents to perform at least 30 cell or tissue assays based on a total reaction volume of 500 µL.

Kit Contents

Component	Concentration	Amount
AFDye 532 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 (dH_2O)
- 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® 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 µM to 5 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 5 µM, 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 µL that would be suitable for a monolayer of adherent cells on an 18 × 18-mm coverslip or for 100 µL 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® 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® X-100 in PBS and incubate for 15 minutes at room temperature.

Material Preparation

AFDye 532 Azide Plus (Component A)	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.
Reaction Buffer (Component B)	To prepare a required amount of **1x reaction buffer**, dilute the appropriate volume from **Reaction Buffer (Component B)** bottle 1:10 with deionized water. Store undiluted 10X reaction buffer at 2–8°C. The 10X solution is stable for 1 year.
Copper Catalysts (Component C)	Ready to use. When stored as directed, this stock solution is stable for up to 1 year.
Reducing Agent (Component D)	Prepare **1 x solution** of **Reducing Agent (Component D)** that is enough for one day. Weight our 20 mg of **Reducing Agent (Component D)** into 2 mL vial, add 1.8 mL of deionized water. Vortex until completely dissolved. *Note-* reducing agent is susceptible to oxidation and turns brown when oxidized. We recommend always using freshly prepared solution of reducing agent.
Wash Buffer (Component E)	Ready to use. When stored as directed, this stock solution is stable for up to 1 year.

Click Labeling Reaction

1. Prepare required amount of **1x solution of Reducing Agent (Component D).** This solution should be used on the same day.
2. For labeling of fixed and permeabilized, cells remove the permeabilization buffer and wash the sample twice with PBS. Remove the wash solution.
3. 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 **Material Preparation**)	435 µL
AFDye 532 Azide Plus (prepared in **Material Preparation**)	10 µL
Copper Catalyst (Component C)	5 µL
1x Solution of Reducing Agent (prepared in **Step 1**)	50 µL

4. **Immediately** add the reaction cocktail to the sample. Evenly distribute the reaction cocktail over the sample.
5. Protect reaction from light and allow click reaction to incubate for 20-30 minutes at room temperature.
6. Remove the reaction cocktail and wash sample once with Wash Buffer (Component E).
7. Remove the Wash Buffer and wash the sample once with PBS.
8. 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.
9. If additional staining is desired, proceed with fixed-cell stains (e.g., nuclear counterstain) following manufacturer's recommendations.
10. The sample is now ready for downstream processing and/or analysis.

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