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EZ-Click™ Plus MB 647 Picolyl Azide Labeling Kit

Product No. 1216

Introduction

EZ-Click™ Plus MB 647 Picolyl Azide Labeling Kit utilizes a copper-chelating azide that dramatically accelerates the Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAC) reaction under conditions relevant to biomolecular labeling. The use of picolyl azide labeling reagent allows the modulation of copper levels in the click reaction by varying the amounts of $CuSO_4$ and copper protectant (chelator), thus minimizing the deleterious effects of copper without sacrificing reaction efficiency. The amount of free copper required for an efficient click reaction is sample-dependent and should be optimized for each sample type. The kit provides all the necessary reagents for at least 30 cell or tissue assays based on a total reaction volume of 0.5 mL.

Kit Contents

Component	Concentration	Amount
MB 647 Picolyl Azide (Component A)	-	150 µg
Reaction Buffer (Component B)	10 x solution	4 mL
Copper (II) Sulfate (Component C)	100 mM	0.5 mL
Copper Protectant (Component D)	1 x solution	0.5 mL
Reducing Agent (Component E)	-	400 mg

Materials Required but Not Provided

- Alkyne-tagged 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 a picolyl azide detection reagent may range from 0.5 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.
- The EZ-Click™ Plus kit allows the modulation of free copper levels in the click reaction by varying the amounts of **$CuSO_4$ (Component C)** and **Copper Protectant (Component D)**, thus minimizing the deleterious effects of copper without sacrificing reaction efficiency. The amount of free copper required for an efficient click reaction is sample-dependent and should be optimized for each sample type.
- 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.

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.
- Remove media from sample and add an appropriate amount 3.7% formaldehyde in PBS. Incubate for 15 minutes at room temperature.
- Remove the fixative and wash sample twice with 3% BSA in PBS.
- Remove the wash solution and add an appropriate amount of 0.5% Triton. X-100 in PBS and incubate for 20 minutes at room temperature

Material Preparation

MB™ 647 Picolyl Azide Detection Reagent (Component A)	Prepare a 250 µM stock solution of the **MB™ 647 picolyl azide (Component A)** by adding 560 µL of anhydrous DMSO to **Component A**, and mix well. After use, store any remaining stock solution at ≤–20°C. When stored as directed, this stock solution is stable for up to 1 year.
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 (II) Sulfate (Component C)	Ready to use. Stable for 1 year when stored at ambient temperature.
Copper Protectant (Component D)	Ready to use. Store unused stock refrigerated at 2-8^{0}C. Stable for 1 year when stored as directed.
Reducing Agent (Component E)	Prepare a **10x stock solution** by dissolve **Reducing Agent (Component E)** in 1.8 mM dH_2O, vortex until completely dissolved. Store unused stock at -20^{0}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. Prepare $CuSO_4$ and copper protectant mix by mixing 5 µl of **Copper (II) Sulfate (Component C)** solution and 5 µL of stock solution of **Copper Protectant (Component D).** 1:1 $CuSO_4$:copper protectent ratio provide optimal level of free copper and copper protectant for most labeling applications. Ratios below or above 1:1 are also possible, and should be optimized per the specific sample type. To achieve higher level of copper protectant prepare a mix by mixing 5 µl of **Copper (II) Sulfate (Component C)** and higher volume (5-30 µL) of **copper protectant (Component D).** To achieve higher level free copper mix 5 µl of **Copper (II) Sulfate (Component C)** and smaller amount (0-5 of µL) of **Copper Protectant (Component D).**
2. Prepare required amount of **1x solution of Reducing Agent (Component D)** by diluting 10x stock solution with dH_2O. This solution should be used on the same day.
3. For labeling fixed and permeabilized cells remove the permeabilization buffer and wash sample twice with 3% BSA in PBS. Remove the wash solution.
4. For each labeling reaction prepare a reaction cocktail in 1.5 mL microfuge tube **in the order given**, and then vortex briefly to mix.

Component	Amount
1x Reaction Buffer (prepared in **Material Preparation**)	430 µL
MB 647 Picolyl Azide stock solution (prepared in **Material Preparation**)	10 µL
$CuSO_4$:copper protectant mix (prepared in **Step 1**)	entire amount prepared
1x solution of Reducing Agent (prepared in **Step 2**)	50 µL

5. **Immediately** add the reaction cocktail to the sample. Evenly distribute the reaction cocktail over the sample.

6. Protect reaction from light and allow click reaction to incubate for 30 minutes at room temperature.
7. Remove the reaction cocktail and wash sample once with 3% BSA in PBS. Remove the wash solution.
8. If additional labelling desired, proceed with fixed-cell stains (e.g., nuclear counterstain) following manufacturer's recommendations.
9. The sample is now ready for downstream processing and/or analysis.