

Click-&-Go™ EdU 568 Imaging Kit

Product No. 1327

Introduction

Click-&-Go™ EdU 568 Imaging Kit is optimized for imaging alkyne-tagged biopolymers with green-fluorescent AFDye 568 Azide (Alexa Fluor® 568 Azide equivalent). The kit includes blue fluorescent Hoechst 33342 dye for performing cell cycle analysis on samples from adherent cells. Sufficient amount of reagents provided for imaging 50 (18x18) coverslips using 500 µL of reaction buffer per test.

Kit contents

Component	Concentration	Amount
AFDye 568 Azide (Component A)	-	1 vial
Reaction Buffer (Component B)	10X solution	4 mL
Copper (II) Sulfate (Component C)	100 mM	1.2 mL
Reducing Agent (Component D)	-	400 mg
Hoechst 33342 (Component E)	10 mg/mL	50 µL

Materials required but not provided

- Fixative (for example 3.7% Formaldehyde in PBS)
- Permeabilization reagent (for example, 0.5% solution of Triton® X-100 in PBS)
- 3% BSA in PBS (pH 7.4)
- Coverslips/microscope slides, mounting media
- PBS buffer, pH 7.4
- Deionized water

Additional information

Hoechst 33342 (Component E) is a known mutagen. Use the dye with appropriate precautions.

Material preparation

AFDye 568 Azide (Component A)

Add 70 μ L of DMSO or water. This solution is stable up to 1 year if stored at -20C protected from light.

Reaction Buffer (Component B)

To prepare a required amount of **1x reaction buffer** (see Table 1), dilute the appropriate volume from **Reaction Buffer (Component B)** bottle 1:10 with deionized water. To convert the entire amount of **10x Reaction buffer** into 1x working solution transfer entire bottle of **10x Reaction Buffer** (4 mL) into 36 mL of deionized water. Store undiluted 10X reaction buffer at 2–8°C. The 10X solution is stable for 1 year.

Reducing Agent (Component D)

Prepare **1 x solution of Reducing Agent (Component D)** that is enough for one day. Weigh out 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 use freshly prepared solution of reducing agent.

Cell fixation and permeabilization

The following protocol is provided for fixation step using 3.7% formaldehyde in PBS followed by a 0.5% Triton[®]X-100 permeabilization step. Protocols using other fixation/permeabilization reagents, such as methanol and saponin also can be used.

- 1.1 Transfer each coverslip into a single well. For convenient processing use 6-well plates.
- 1.2 After metabolic labeling, remove the media and add 1 mL of 3.7% formaldehyde in PBS to each well containing the coverslips. Incubate for 15 minutes at room temperature
- 1.3 Remove the fixative and wash the cells in each well twice with 1 mL of 3% BSA in PBS.
- 1.4 Remove the wash solution. Add 1 mL of 0.5% Triton[®] X-100 in PBS to each well, then incubate at room temperature for 20 minutes.

Alkyne detection

Note: 500 μ L of the reaction cocktail is used per coverslip. A smaller volume can be used as long as the remaining reaction components are maintained at the same ratios.

- 2.1 Weigh out 20 mg of **Reducing Agent (Component D)** into 2 mL vial, add 1.8 mL of deionized water, vortex until completely dissolved. This solution should be used freshly prepared on the same day.
- 2.2 Prepare required amount of the reaction cocktail according to Table 1. **Add the ingredients in the order listed in the table.** Use the reaction cocktail within 15 minutes of preparation.

Table 1.

Reaction component	Number of coverslips						
	1	2	4	5	10	25	50
1x Reaction Buffer (Material preparation)	430 µL	860 µL	1.8 mL	2.2 mL	4.3 mL	10.7 mL	21.4 mL
Copper (II) Sulfate (Component C)	20 µL	40 µL	80 µL	100 µL	200 µL	500 µL	1 mL
AFDye Azide solution (Material preparation)	1.2 µL	2.5 µL	5 µL	6 µL	12.5 µL	31 µL	62 µL
1x Reducing Agent (step 2.1)	50 µl	100 µL	200 µL	250 µL	500 µL	1.25 mL	2.5 mL
Total Volume	500 µL	1 mL	2.5 mL	5 mL	7.5 mL	15 mL	25 mL

- 2.3 Remove the permeabilization buffer (step 1.4).
Wash the cells in each well twice with 1 mL of 3% BSA in PBS.
Remove the wash solution.
- 2.4 Add 0.5 mL of the **Reaction Cocktail** to each well containing a coverslip.
Rock the plate briefly to insure that the reaction cocktail is distributed evenly over the coverslip.
- 2.5 **Protect from light**, and incubate the plate for 30 minutes at room temperature.
- 2.6 Remove the reaction cocktail.
Wash each well once with 1 mL of 3% BSA in PBS.
Remove the wash solution.

At this point the samples are ready **DNA staining**. If no **DNA staining** is desired, proceed to **Imaging**.

If antibody labeling of the samples is desired, proceed to labeling according to manufacturer's recommendations. **Keep the samples protected from light during incubation.**

DNA staining

- 3.1 Wash each well with 1 mL of PBS.
Remove the wash solution.
- 3.2 Prepare 1x **Hoechst 33342 solution** by diluting stock solution of **Hoechst 33342 (Component E)** 1:2000. The final concentration of 1x **Hoechst 33342 solution** is 5 µg/mL.

Final concentrations of 1x **Hoechst 33342** may range from 2 µg/mL to 10 µg/mL.
- 3.3 Add 1 mL of 1x **Hoechst 33342 solution** per well.
Protected from light.
Incubate for 30 minutes at room temperature.

- 3.4 Remove the Hoechst 33342 solution.
- 3.5 Wash each well twice with 1 mL of PBS.
- 3.6 Remove the wash solution.

Imaging

Labeled cells are compatible with all method for slide preparation. See **Table 2** for approximate fluorescence excitation/emission maxima for AFDye 568 and Hoechst 33342 dye bound to DNA.

Table 2.

	Excitation (nm)	Emission (nm)
AFDye 568	578	602
Hoechst 33342 bound to DNA	350	461