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Click-&-Go™ EdU 647 Flow Cytometry Kit

Product No. 1391

Introduction

Click-&-Go™ EdU 647 Flow Cytometry Kit is optimized for imaging alkyne-tagged biopolymers with far-red-fluorescent AFDye 647 Azide using standard flow cytometry methods. The kit contains all components needed to detect the incorporated alkynes, including aldehyde-based fixation and detergent permeabilization reagents. Sufficient amount of reagents provided for 50 assays based on the protocol provided.

Kit contents

Component	Concentration	Amount
AFDye 647 Azide (Component A)	-	-
Fixative (Component B)	4% PBS solution	5 mL
Copper (II) Sulfate (Component C)	100 mM	0.55 mL
Reducing Agent (Component D)	NA	400 mg
Saponin-based permeabilization and wash reagent (Component E)	10 x	50 mL

Materials required but not provided

- 1% BSA in PBS (pH 7.4)
- Flow cytometry tubes
- PBS buffer, pH 7.4
- Deionized water
- DMSO

Additional information

- Fixative (Component B) contains paraformaldehyde, which is harmful. Use with appropriate precautions.
- Saponin-based permeabilization and wash reagent (Component E) contains sodium azide, which yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

Material preparation

AFDye 647 Azide (Component A)	Add 140 µL of DMSO or water. This solution is stable up to 1 year if stored at -20C protected from light.
1X saponin-based permeabilization and wash reagent (Component E)	To prepare 500 mL of 1X saponin-based permeabilization and wash reagent, add 50 mL of **Component E** to 450 mL of 1% BSA in PBS. Smaller amounts can be prepared by diluting a volume of **Component E** 1:10 with 1% BSA in PBS. After use, store any remaining solutions at 2–8°C. When stored as directed, the 1X solution is stable for 6 months and the 10X solution is stable for 12 months after receipt.
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 mL of deionized water. Vortex until completely dissolved. Note- reducing agent is susceptible to oxidation and turns brown when oxidized. If solution appears brown do not use.

Alkyne labeling

Please visit Technical Resources section of our website to download a protocol for labeling cells with alkyne-containing matabolic labeling.

Optional cell-surface antigen staining with antibodies

1.1 After alkyne labeling wash cells once with 3 mL of 1% BSA in PBS, pellet cells by centrifugation, and remove supernatant.

1.2 Dislodge the pellet and resuspend cells at 1×10^7 cells/mL in 1% BSA in PBS.

1.3 Add surface antibodies, mix well and incubate for the recommended time and temperature protected from light.

1.4 Proceed to cell fixation step.

Cell fixation and permeabilization

The following protocol is provided for fixation step using saponin-based permeabilization and wash reagent. This reagent can be used with whole blood or cell suspensions containing red blood cells, as well as with cell suspensions containing more than one cell type. This permeabilization and wash reagent maintains the morphological light scatter characteristics of leukocytes while lysing red blood cells. Protocols using other fixation/permeabilization reagents, such as methanol and 3.7% formaldehyde in PBS followed by a 0.5% Triton®X-100 permeabilization step also can be used.

2.1 Wash the cells once with 3 mL of 1% BSA in PBS, pellet the cells, and remove the supernatant.

2.2 Dislodge the pellet, add 100 µL of Fixative (Component B) and mix well, and incubate for 15 minutes.

2.3 Remove the fixative and wash the cells in each with 1 mL of 1% BSA in PBS. If red blood cells or hemoglobin are present in the sample repeat the wash step. If require remove all residual cell debris.

2.4 Remove the wash solution and resuspend the cells in 100 µL of 1X saponin-based permeabilization and wash reagent, and mix well. Incubate the cells for 15 minutes.

2.5 Proceed directly to alkyne detection step or to treating cells with antibodies to intracellular antigens followed by EdU detection step.

Alkyne detection

Note: 500 µL is used per reaction. A smaller volume can be used as long as the remaining reaction components are maintained at the same ratios.

3.1 Weight our 20 mg of **Reducing Agent (Component D)** into 2 mL vial, add 1 mL of deionized water, vortex until completely dissolved. This solution should be used freshly prepared on the same day.

3.2 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 reactions					
	1	**2**	**5**	**10**	**15**	**50**
PBS buffer	438 µL	875 µL	2.19 mL	4.38 mL	6.57 mL	21.9 mL
Copper + Protectant (Component C)	10 µL	20 µL	50 µL	100 µL	150 µL	500 µL
AFDye 647 Azide solution (Material preparation)	2.5 µL	5 µL	12.5 µL	25 µL	37.5 µL	125 µL
1x Reducing Agent (step 3.1)	50 µl	100 µL	250 µL	500 µL	750 µL	2.5 mL
Total Volume	500 µL	1 mL	2.5 mL	5 mL	7.5 mL	25 mL

3.3 Remove the permeabilization buffer (step 2.5).

3.4 Add 0.5 mL of the **Reaction Cocktail** to each tube. Rock the tube briefly to insure that the reaction cocktail is distributed evenly.

3.5 **Protect from light**, and incubate the tube for 30 minutes at room temperature.

3.6 Remove the reaction cocktail.
Wash the cells well once with 3 mL of 1X saponin-based permeabilization and wash reagent.
Pallet the cells and remove the wash solution.

3.7 If intracellular antibody labeling required dislodge the cell pellet and resuspend the cells in 100 µL of 1X saponin-based permeabilization and wash reagent.

3.8 If no intracellular antibody labeling required add 500 µL of 1X saponin-based permeabilization and wash reagent.

At this point the samples are ready for **DNA staining**. If no **DNA staining** is desired, proceed to analyzing the cells on a flow cytometer.

Optional intracellular or surface antigens staining

4.1 Add antibodies against intracellular antigens or against surface antigens that use RPE, PR-tandem or Quantum Dot antibody conjugates. Mix well.

4.2 Protect from light and incubate the tubes for the time and temperature required for antibody staining.

4.3 If **Alkyne Detection** has not yet performed, wash the cells once with 3 mL of 1% BSA in PBS, pellet the cells, and remove the supernatant. Dislodge the cell pellet and add 100 µL of 1x saponin-based permeabilization and wash reagent, mix well and proceed to **Alkyne Detection** (step 3.1)

4.4 If **Alkyne Detection** has been performed (steps 3.1–3.8), wash each tube with 3 mL of 1X saponin-based permeabilization and wash reagent, pellet the cells, and remove the supernatant. Dislodge the cell pellet and resuspend the cells in 500 µL of 1X saponin-based permeabilization and wash reagent, and proceed to DNA staining, or to the cell analysis on a flow cytometer.

Optional DNA staining

5.1 Add the appropriate DNA stain to each tube and mix well.

5.2 Protect from light and incubate as recommended by a manufacture.

5.3 Remove the DNA stain and wash each tube with 3 mL of 1X saponin-based permeabilization and wash reagent each well.

Flow Cytometry Analysis

Use a low flow rate during acquisition, if a traditional flow cytometer with a hydrodynamic focusing is used to measure the total DNA content. The same collection rate and cell concentration should be used for each sample within an experiment. Detect the fluorescent signal generated by DNA content stains with linear amplification. The fluorescent signal generated by labeling is best detected with logarithmic amplification.

The excitation and emission maxima of AFDye 647 are 650 nm and 671 nm.