CLICK CHEMISTRY TOOLS

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EdU Imaging, Flow Cytometry

This protocol was tested with A549, HeLa, and NIH/3T3 cells but it can be adapted for any adherent cell type. In initial experiments, we recommend starting with 1.5 µM final concentration of an azide detection reagent. Slight adjustment of an azide detection reagent concentration might be required in case of high background or low signal intensity.

Azide detection reagents, and EdU can be ordered from Click Chemistry Tools.

Prepare the following reagents:

- 100 mM copper sulfate in water
- 100 mM sodium ascorbate in water (20 mg/mL, should be used freshly prepared)
- 300 µM azide labeling reagent in water or DMSO
- Fixative (for example 3.7% Formaldehyde in PBS)
- Permeabilization and wash reagent (for example, saponin-based permeabilization and wash reagent)
- 1% BSA in PBS (pH 7.4)
- PBS buffer, pH 7.4
- Deionized water

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.

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

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

1.3 Remove the fixative and wash the cells 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.

1.4 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.

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

EdU detection

Note: 500 µL 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 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**	**5**	**10**	**15**	**50**
PBS buffer	438 µL	875 µL	2.19 mL	4.38 mL	6.57 mL	21.9 mL
Copper Sulfate	10 µL	20 µL	50 µL	100 µL	150 µL	500 µL
Azide solution	2.5 µL	5 µL	12.5 µL	25 µL	12.5 µL	125 µL
Sodium Ascorbate	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

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

2.3 **Protect from light**, and incubate the plate for 30 minutes at room temperature.

2.4 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.

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

2.6 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.