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http://www.clickchemistrytools.com
E: inquiries@clickchemistrytools.com
T: 480 584 3340
F: 866 717 2037

Labeling cells with EdU

The following is a general protocol was tested with several cell lines including A549, HeLa, and NIH/3T3 cells, but it can be used for any adherent cell type. Growth medium, cell density, cell type variations, and other factors may influence labeling. We recommend 10 µM EdU concentration as initial experimental conditions followed by testing a range of EdU concentrations to determine the optimal concentration for your cell type and experimental conditions.

1. Plate the cells on coverslips at the desired density and allow them to recover overnight before additional treatment.
2. Prepare 20 mM solution of EdU in DMSO or water (use 1.98 mL of solvent per 10 mg of EdU)
3. Add desired amount of EdU to cells in culture medium to achieve 10 µM final EdU concentration. We have found a final concentration of 10 µM EdU to be sufficient for labeling of most cell lines.
4. During addition of EdU to cells in culture, avoid disturbing the cells in any way (eg, centrifugation steps or temperature changes) that may disrupt the normal cell cycling patterns. The cell culture density should not exceed 2×10^6 cells/mL.
5. Incubate the cells for the desired length of time under conditions optimal for the cell type. Different cell types may require different incubation periods for optimal labeling with EdU. As a starting point, we recommend 10 µM EdU for 1 hour.
6. Harvest cells, pellet by centrifugation, and remove EdU-containing media.
7. Cells are ready for fixation and permeabilization followed by EdU imaging.