Introduction

Changes in cell proliferation as measured by the incorporation of nucleoside analog into actively synthesizing DNA have been the basis for assessing treatments which alter or block phases of the cell cycle. Initially radioactive thymidine was used and later replaced by bromo-deoxyuridine (BrdU) incorporation. We introduce a new method for a direct measurement of the S-phase fraction through the use of click chemistry. Here, a labeling strategy using the Click-iT™ EdU cell proliferation assay from Molecular Probes®, demonstrates that cell proliferation and cell cycle blockers can be assessed by flow cytometry.

Click chemistry, originally independently defined by Sharpless and Meldal, has been adapted to measuring cell proliferation through the direct detection of nucleoside incorporation. The assay uses the incorporation of an alkyne modified nucleoside supplied in the growth medium for a defined time period.

Cells are then fixed, permeabilized, and reacted with a dye labeled azide, catalyzed by copper(I). A covalent bond is formed between the dye and the incorporated nucleoside, and the fluorescent signal can then be measured by flow cytometry, or high content screening (HCS).

EdU (5-ethyl-2’-deoxyuridine) based S-phase measurement offers distinct advantages over antibody-based BrdU method. Compared to BrdU, the click chemistry approach requires less time and sample preparation, and provides greater reliability with at least equivalent sensitivity.

Results and Conclusions

A novel method of measuring cell proliferation by nucleoside incorporation is presented using click chemistry based cycloaddition reaction between an alkyne and an azide.

The thymidine analog EdU provides a terminal alkyne and reacts with a fluorescent compound to form a covalent bond, thus labeling replicating DNA.

A simplified workflow enables accurate, consistent performance—no denaturation steps, no harsh treatments.

Click-iT™ EdU Alexa Fluor® 488 azide dye can be multiplexed with antibody based detection.

Cell cycle arrest drugs can be screened using this simple and rapid protocol, compatible with flow cytometry, imaging, and high content screening platforms.

LIVE/DEAD® Flexible Dead Cell Stains can be used for gating out dead cell populations.

EdU labeling has been demonstrated in both adherent and suspension cell lines, including Jurkat, MOLT4, HeLa, COS7, CHOK1, A549, 3T3, and in phorbol stimulated peripheral blood lymphocytes.

References


References


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