DETECTION OF S-PHASE CELL CYCLE PROGRESSION USING 5-ETHYNYL-2'-DEOXYURIDINE INCORPORATION WITH CLICK CHEMISTRY

Jolene A. Bradford1, Scott T. Clarke1, Suzanne B. Buck1, Dani Hill1, Yih-Tai Chen1, Kyle Gee1, Brian Agnew1, Adrian Salic2
1Molecular Probes® - Invitrogen Detection Technologies • 29851 Willow Creek Rd • Eugene, Oregon 97402 • USA
2Harvard Medical School, Boston MA • USA

Introduction

Labeling, detection, and quantification of cells in the synthesis phase of cell cycle progression are not only important in characterizing basic biology, but also in defining the cellular responses to drug treatments. S-Phase has classically been assessed with incorporation of either radioactive thymidine or 5-bromo-2'-deoxyuridine (BrdU). We introduce the novel use of click chemistry for the labeling and detection of DNA synthesis. This labeling strategy uses the incorporation of the thymidine analog 5-ethynyl-2'-deoxyuridine (EdU), followed by chemical coupling of the analog with an azide conjugated fluorophore for detection. This protocol uses standard aldehyde fixation and detergent permeabilization, avoiding the need for denaturation steps required for BrdU labeling. Results using click chemistry detection of EdU are shown using flow cytometry, fluorescence microscopy and automated fluorescence microscopy (HCS) platforms. Comparison data of EdU and BrdU detection are shown. Results demonstrate that click chemistry-based detection of incorporated EdU provides a rapid and accurate assessment of S-phase using standard fixation and permeabilization protocols. Results obtained with EdU detection, without having to denature the DNA, are equivalent to results obtained with BrdU detection. The novel method using click chemistry-based detection of EdU can be used to measure S-phase cell cycle progression in multiple platforms.

Results and Conclusions

Click chemistry is employed in this application because the reaction between an alkyne and an azide is bioorthogonal; it occurs selectively, without the interference of any other fundamental groups present within complex biological systems. Alkyne and azide compounds are stable, inert functional groups which typically are not present in biological systems.

Figure 1 – Click Chemistry Based Detection of Metabolically Incorporated DNA Analog

[Diagrams and graphs showing click chemistry reaction and detection results]

Figure 5 – Determination of S-phase % with Click-iT™ EdU

[Graph showing S-phase percentage determined using Click-iT™ EdU]

Figure 6 – Acid denaturation comparison of EdU to BrdU labeling.

[Graph comparing EdU and BrdU labeling with and without acid denaturation]

Figure 7 – Comparison of EdU to BrdU detection using EdU method - paraformaldehyde fixative & saponin permeabilization

[Graph comparing EdU and BrdU detection with and without saponin permeabilization]

Figure 8 – Imaging with EdU Incorporated Cells Labeled with Click-iTTM Proliferation kit

[Images showing EdU labeled cells with click chemistry labeling]

Figure 9 – HeLa cells were grown in 10 µM EdU for 2 hours and labeled with Click-iTTM EdU Alexa Fluor® 488 (green), a tubulin/GAM Alexa Fluor® 555 conjugate (red), and a nuclear stain (DAPI, blue).

[Images of HeLa cells labeled with EdU and DAPI]

Results and Conclusions

• A novel method of measuring cell proliferation by nucleoside incorporation using click chemistry detection is presented.
• The thymidine analog EdU incorporates into DNA, thus providing a terminal alkyne which reacts with an azide labeled with a fluorescent compound to form a covalent bond, thus labeling synthesizing DNA.
• Click chemistry detection of incorporated EdU uses standard fixation and permeabilization protocols.
• No denaturation of DNA is required for EdU detection using click chemistry.
• A simplified protocol enables:
  • accurate, consistent performance
  • shorter time to answer, basic assay is complete in 2 hours
  • comprehensive data, compatible with multiplexing
  • The Click-iTTM EdU cell proliferation assay can be used with flow cytometry, imaging, and automated imaging platforms.

References