Improved Click Chemistry Demonstrating EdU Cell Proliferation with GFP-expressing Cells and R-PE-based Immunophenotyping

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ABSTRACT

The current Click-iT® EdU kit for direct S-phase determination cannot be used for the simultaneous detection of GFP and R-PE fluorescence due to the presence of copper and reactive oxygen species mediated damage to fluorescent proteins. We present modifications to the click reaction resulting in "copper safe" catalysis of the click reaction. Flow cytometry measurements of cell proliferation using Click-iT® EdU are demonstrated to be compatible with immunophenotyping panels using R-PE conjugates and GFP-expressing cell lines. The basis of the click chemistry improvement is the use of a copper (I) ligand combined with a modified dye azide detection reagent. We optimized components in the improved click reaction and found those that best preserved R-PE fluorescence while obtaining a bright EdU signal. Jurkat cells were pulsed with EdU, labeled with R-PE conjugated antibodies and then fixed and permeabilized. EdU incorporation was then detected using click chemistry demonstrating improved detection while maintaining R-PE signal. Additionally, cells expressing GFP were pulsed with EdU and used to demonstrate GFP and click chemistry compatibility. The modified click reaction is an improvement over the original copper based click reactions and will further enhance the utility of EdU based cell proliferation applications in multi-color flow cytometry.

INTRODUCTION

EdU (ethynyl-deoxyuridine) incorporation into nascent DNA detected with copper-catalyzed click chemistry (CuAAC) as a method for measuring cell proliferation was first described in 2008.1 This method reduces assay time and improves the work flow compared to the traditional method using antibody-based BrdU detection. However, copper and reactive oxygen species (ROS) mediated damage to fluorescent proteins prevents the simultaneous detection of EdU, GFP or Rhodamine (R-PE) fluorescence. We present chemical modifications to the click reaction resulting in "copper safe" catalysis of the click reaction, whereby GFP and R-PE fluorescence are preserved while EdU based cell proliferation is detected. The basis of the improvement is the use of a copper (I) ligand combined with a modified dye azide detection reagent. Together, the copper ion is sequestered with the ligand and prevented from damaging protein fluorescence but still remains available to catalyze the click reaction. The use of the described modified click reaction is an improvement over earlier described click reactions and will further enhance the utility of EdU based cell proliferation assays by facilitating multiplexing using common fluorescent proteins.

MATERIALS AND METHODS

To study the effects of our modified reactants on GFP fluorescence, we used an A375 cell line constitutively expressing GFP-SRFQ under control of a CMV promoter. ERK2 A375 cells were pulsed with 10 μM EdU for 2 hours prior to fix/perm and click labeling with modified Alexa Fluor® 647 azide. For R-PE immunophenotyping experiments Jurkat cells were pulsed with 10 μM EdU for 2 hours prior to surface staining with FITC-DOP and click labeling with modified Alexa Fluor® 488 azide. We identified the click reaction conditions that best preserved GFP and R-PE fluorescence while obtaining a bright EdU signal. Quintessential cell cycle bi-variant plots were generated using Jurkat cells pulsed with 10 μM EdU for 2 hours prior to fix/perm then click labeled with modified Alexa Fluor® 647 azide and DNA content cell cycle stained with FxCycle™ Violet. All flow cytometry data was collected and analyzed on the Attune® Acoustic Focusing Cytometer.

Figure 1 – Click chemistry-based detection of metabolically incorporated DNA analog EdU

Figure 2 – Use of the Click-iT® EdU imaging kit with proliferating A375 cells

Figure 3 – Use of modified click components preserves GFP fluorescence while maintaining a robust EdU signal

Figure 4 – Use of modified click components preserves R-PE fluorescence while maintaining a robust EdU signal

Figure 5 – Comparison of classic Click-iT® EdU and modified Click-iT® EdU in the determination of S-phase percent

CONCLUSIONS

The use of an optimized ratio of chelator to copper combined with a modified dye azide creates an ideal click reaction that sequesters Cu ions and protects GFP and R-PE fluorescence. These breakthroughs enable scientists to use the Click-iT® EdU cell proliferation technology with GFP-expressing cell lines and in conjunction with immunophenotyping panels containing R-PE.

REFERENCES

2. S. B. Buck et al., Biotechniques 43, 527 (Oct, 2007).

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