

Technical Tip: Conversion of the Click-iT™ EdU High-Throughput Imaging (HCS) Assay for Conventional Fluorescence Microscopy

Converting the Click-iT™ EdU Alexa Fluor® High-Throughput Imaging (HCS) Assay for use with imaging cells on coverslips is easily accomplished without the need for any additional steps or reagents. Simply follow the protocol provided in the product information sheet, MP10027, but adjust volumes of the Click-iT™ reaction cocktail and wash buffers as described below and in Table 1.

The Click-iT™ EdU Alexa Fluor® High-Throughput Imaging (HCS) Assay *2-plate size* contains sufficient EdU to label 100 mL media at a 10 µM concentration and provides enough detection reagents for 50 coverslips using the protocol described below. Additional EdU is available separately from Invitrogen (Cat. no. A10044).

Preparing the Stock Solutions

Prepare the reagents as described in section 1.1–1.5 of MP10027.

Labeling Cells with EdU

The following protocol was developed for adherent cells.

- 2.1 Prepare a 2X working solution of EdU (Component A) in complete medium from the 10 mM stock solution. A suggested starting concentration is 10 µM.
- 2.2 Prewarm the 2X EdU solution, then add an equal volume of the 2X EdU solution to the volume of media containing cells to be treated to obtain a 1X EdU solution (e.g., for a final concentration of 10 µM, replace half of the media with fresh media containing 20 µM EdU). Replacement of all of the media is not recommended since this could affect the rate of cell proliferation.
- 2.3 Incubate under conditions optimal for cell type for the desired length of time. The choice of time points and length of time depends on the cell growth rate. The time of EdU exposure to the cells allows for direct measurement of cells synthesizing DNA.

Cell Fixation and Permeabilization

Transfer the coverslips into a 6-well plate for convenient processing such that each well contains a single coverslip.

- 3.1 After incubation, remove media and add 1 mL 3.7% formaldehyde in PBS to each well containing the coverslips. Incubate for 15 minutes at room temperature.
- 3.2 Remove fixative and wash cells in each well twice with 1 mL 3% BSA in PBS.
- 3.3 Remove the wash solution. Add 1 ml 0.5% Triton® X-100 in PBS to each

well and incubate for 20 minutes at room temperature.

EdU Detection

4.1 Prepare 1X Click-iT™ EdU buffer additive by diluting the 10X solution 1:10 in deionized water. Prepare this solution **fresh** and use the solution on the same day.

4.2 Prepare Click-iT™ reaction cocktail according to Table 1, below.

Note: Use the Click-iT™ reaction cocktail within 15 minutes of preparation.

4.3 Remove the permeabilization buffer (step 3.3) and wash cells in each well twice with 1 mL 3% BSA in PBS. Remove the wash solution.

4.4 Add 0.5 mL Click-iT™ reaction cocktail to each well containing a coverslip. Rock the plate briefly to insure that the reaction cocktail is distributed evenly over the coverslip.

Note: You may need to adjust the amount of dye azide to provide optimum signal to background.

4.5 Incubate for 30 minutes at room temperature, protected from light.

4.6 Remove the reaction cocktail and wash each well once with 1 mL 3% BSA in PBS. Remove the wash solution.

Table 1. Click-iT™ reaction cocktails.

Reaction Components	Number of Coverslips						
	1	2	4	5	10	25	50
1X Click-iT™ reaction buffer (prepared in step 1.4)	430 µL	860 µL	1.8 mL	2.2 mL	4.3 mL	10.7 mL	21.4 mL
CuSO ₄ (Component H)	20 µL	40 µL	80 µL	100 µL	200 µL	500 µL	1 mL
Alexa Fluor® azide (prepared in step 1.3)	1.2 µL	2.5 µL	5 µL	6 µL	12.5 µL	31 µL	62 µL
Reaction buffer additive (prepared in step 4.1)	50 µL	100 µL	200 µL	250 µL	500 µL	1.25 mL	2.5 mL
Total volume	500 µL	1 mL	2 mL	2.5 mL	5 mL	12.5 mL	25 mL

Antibody Staining (optional) and DNA Staining

Proceed with the optional antibody or nuclear staining using steps 5.1–5.8 and 6.1–6.4 respectively, as described in MP10027.

Adjust the volumes of the primary and secondary antibodies, accordingly. You may need to perform additional wash steps with a larger volume to reduce non-specific antibody background.

Imaging and Analysis

Click-iT™ EdU cells are compatible with all methods of slide preparation including wet mount or prepared mounting media.

See Table 2 for the approximate fluorescence excitation/emission maxima for Alexa Fluor® dyes and Hoechst 33342 dye bound to DNA.

Table 2. Approximate fluorescence excitation/emission maxima.

Fluorophore	Excitation (nm)	Emission (nm)
Alexa Fluor® 488	495	519
Alexa Fluor® 594	590	615
Alexa Fluor® 647	650	670
Hoechst 33342, bound to DNA	350	461

Product List Current prices are available from www.invitrogen.com or from our Customer Service Department

Catalog no.	Product Name	Unit Size
A10027	Click-iT™ EdU Alexa Fluor® 488 High-Throughput Imaging (HCS) Assay *2-plate size*.....	1 kit
A10044	EdU (5-ethynyl-2'-deoxyuridine).....	50 mg
A10208	Click-iT™ EdU Alexa Fluor® 647 High-Throughput Imaging (HCS) Assay *2-plate size*.....	1 kit
A10209	Click-iT™ EdU Alexa Fluor® 594 High-Throughput Imaging (HCS) Assay *2-plate size*.....	1 kit
I36933	Image-iT® FX signal enhancer.....	10 mL
P36930	ProLong® Gold antifade reagent.....	10 mL
P36931	ProLong® Gold antifade reagent with DAPI.....	10 mL
S36936	SlowFade® Gold antifade reagent.....	10 mL
S36938	SlowFade® Gold antifade reagent with DAPI.....	10 mL
14190-144	Dulbecco's Phosphate Buffered Saline (D-PBS) (1X), liquid, without Calcium, Magnesium, or Phenol Red.....	500 mL
14190-250	Dulbecco's Phosphate Buffered Saline (D-PBS) (1X), liquid, without Calcium, Magnesium, or Phenol Red.....	10 × 500 mL

Contact Information

Molecular Probes, Inc.

29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:

6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:

Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:

8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probetech@invitrogen.com

Invitrogen European Headquarters

Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Support: euotech@invitrogen.com

For country-specific contact information, visit www.invitrogen.com

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East, contact our office in Paisley, United Kingdom. All others, contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License No. 223: Labeling and Detection Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2007, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.