Click-iT™ EdU Colorimetric IHC Detection Kit

Catalog No. C10644

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Table 1. Contents and storage

Material	Amount	Concentration	Storage
EdU (5-ethynyl-2'-deoxyuridine) (Component A)	10 mg	N/A	• 2–8°C • Desiccate • Protect from light • DO NOT FREEZE
Click-iT™ EdU Reaction Buffer (Component B)	4 mL	10X solution in Tris-buffered saline	
Copper II sulfate ($CuSO_4$) (Component C)	1 mL	100 mM aqueous solution	
Biotin azide (Component D)	43 µg	N/A	
Dimethylsulfoxide (DMSO) (Component E)	200 µL	N/A	
Click-iT™ EdU Reaction Buffer Additive (Component F)	400 mg	N/A	
Trypsin-EDTA (Component G)[1]	20 mL	1X solution	
Click-iT™ EdU Wash Buffer (Component H)	60 mL	20X solution	
Streptavidin-Peroxidase Conjugate (Component I)	10 mL	1X solution	
DAB Substrate Buffer (Component J)	20 mL	1X solution	
DAB Chromogen (Component K)	1 mL	40X reagent	
Wash chamber[2]	1 unit	N/A	Room temperature

[1] **IMPORTANT!** Upon receipt, aliquot the vial of Trypsin-EDTA (Component G) and store at –20°C.

[2] The wash chamber has been included in the kit for your convenience. We recommend using it for Step 3.5.

Number of assays: Sufficient material is supplied for 50 tissue sections based on the protocol described in this user guide.

N/A: Not applicable.

Introduction

Measuring a cell's ability to proliferate is a fundamental method for assessing cell health, determining genotoxicity, and evaluating anti-cancer drug research. The most accurate method is the direct measurement of DNA synthesis. Initially, this was assayed by the incorporation of radioactive nucleosides such as ^{3}H-thymidine. This method was later replaced by antibody-based detection of the nucleoside analog bromo-deoxyuridine (BrdU). The BrdU nucleoside thymidine analog is incorporated into newly synthesized DNA. After incorporation, the target tissue is isolated, fixed using formalin, and then embedded in paraffin. Detection of the proliferating cells within the tissue section requires DNA denaturation (typically using HCl, heat, or digestion with DNase) to expose the BrdU so that it may be detected with an anti-BrdU antibody (Figure 1, left panel). However, the denaturation step for the BrdU protocol can destroy cell morphology and antigen recognition sites.

The Click-iT™ EdU Colorimetric IHC Detection Kit is a novel alternative to the BrdU assay. EdU (5-ethynyl-2'-deoxyuridine) is a nucleoside analog of thymidine and is incorporated into DNA during active DNA synthesis[1]. After the target tissue is fixed and embedded in paraffin, a click reaction covalently attaches the biotin-azide to the alkyne group on the incorporated EdU. Next, Streptavidin-Peroxidase (horseradish peroxidase) is added to the sample and attaches to the biotin group. Finally, addition of the DAB (peroxidase) substrate results in the colorimetric detection of proliferating cells (Figure 1, right panel).

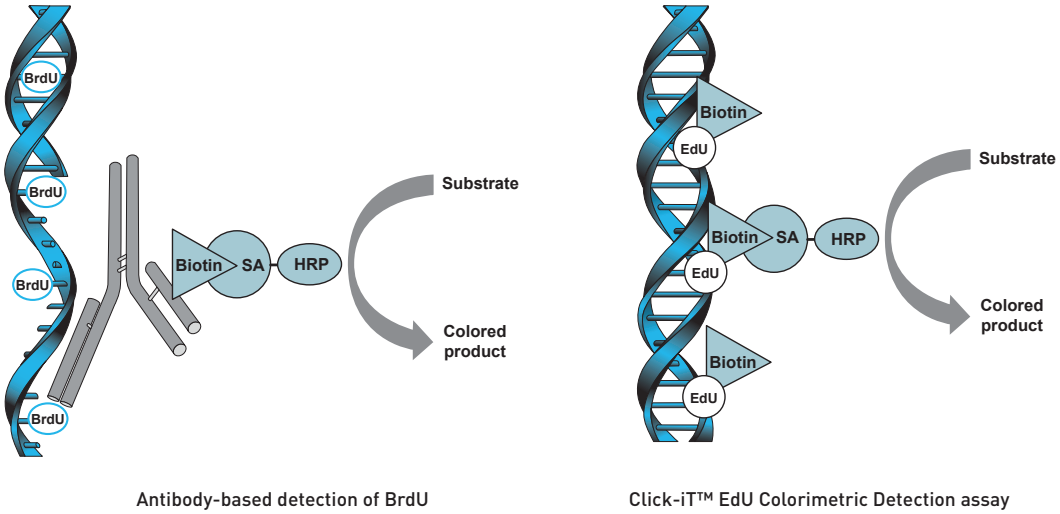

Figure 1. Detection of cell proliferation using the BrdU incorporation method (left panel) or the Click-iT™ EdU Colorimetric Detection assay (right panel).

The small size of the biotin-azide used in the assay allows for efficient detection of the incorporated EdU using mild conditions, and deparaffinization is sufficient for the Click-iT™ EdU detection reagent to gain access to the DNA. However, trypsin digestion is required for the Streptavidin-Peroxidase to access the biotin-tagged EdU. This is in contrast to BrdU assays, which require harsh methods such as HCl denaturation and/or heat inactivation epitope retrieval (HIER).

Additionally, unlike the BrdU assay, which relies upon antibodies that can exhibit non-specific binding, the Click-iT™ EdU Colorimetric IHC Detection Assay utilizes a bioorthogonal (biologically unique) moiety, which results in low background signal, high detection sensitivities, and no cross reactivity issues.

The Click-iT™ EdU Colorimetric IHC Detection Assay Kit contains all of the components needed to detect incorporated EdU present in FFPE (formalin-fixed, paraffin embedded) tissue samples. The kit includes sufficient reagents for labeling 50 tissue sections using 500 µL reaction volume per test.

Before you begin

Materials required but not provided

- 1X Phosphate buffered saline (PBS) (Cat. No. 14190-144 or 14190-250)
- 3% H_2O_2 in PBS
- 18 × 18-mm coverslips (for standard microscopy)
- *Optional*: EdU (Cat. Nos. A10044, E10187, E10415).

Note: The kit includes sufficient reagents for labeling 50 tissue sections using 500 µL reaction volume per test. Larger amounts of EdU may be purchased separately, if required.

Storage and handling

Upon receipt, store the kit components as described until required for use. For optimal storage conditions for each component, see labels on individual components.

IMPORTANT! Upon receiving the vial of Trypsin-EDTA (Component G), aliquot and store at –20°C.

Cautions

DMSO (Component E), provided as a solvent in this kit, is known to facilitate the entry of organic molecules into tissues and is hazardous. Avoid contact with skin and eyes and do not swallow. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of the reagents in compliance with all pertaining local regulations.

Prepare solutions

Allow vials to warm to room temperature before opening.

1.1 EdU is readily soluble in DMSO, alcohol, water, or aqueous buffers. Depending on your application, prepare an appropriate stock solution of EdU in DMSO or aqueous buffer. To make a 10 mM solution of EdU (Component A), add 4 mL DMSO or aqueous solution (i.e., buffer, saline) to Component A and mix well. You can store the 10 mM EdU stock solution at ≤–20°C for up to 1 year.

EdU has a characteristic 288 nm absorption peak which can be used to accurately quantitate stock solutions by absorbance using the extinction coefficient of 12,000 $cm^{-1}M^{-1}$ in methanol. A 10 mg/mL solution (39.6 mM) when diluted 1:1,000 in methanol gives an absorbance of 0.475 at 288 nm.

1.2 **1X Click-iT™ EdU Reaction Buffer (Component B) working solution:** Transfer all of the solution (4 mL) in the Component B vial to 36 mL of deionized water. Rinse the Component B vial with some of the diluted Click-iT™ EdU Reaction Buffer to ensure the transfer of all of the 10X concentrate.

To make smaller amounts of 1X Click-iT™ EdU Reaction Buffer, dilute volumes from the Component B bottle 1:10 in deionized water. After use, store any remaining 1X solution at 2–8°C. When stored as directed, this 1X solution is stable for up to 6 months.

1.3 10X Click-iT™ EdU Reaction Buffer Additive (Component F) stock solution: Add 2 mL of deionized water to the Component F vial, then mix until fully dissolved. After use, store any remaining stock solution at ≤–20°C. When stored as directed, this stock solution is stable for up to 1 year. If the solution develops a brown color, it has degraded and should be discarded.

1.4 Biotin azide (Component D) stock solution: Add 70 µL of DMSO (Component E) to the Component D vial and mix well. After use, store any remaining stock solution at ≤–20°C. When stored as directed, this stock solution is stable for up to 1 year.

1.5 1X Click-iT™ EdU Wash Buffer: Dilute the Click-iT™ EdU Wash Buffer (Component H) 1:20 into 1X PBS.

Note: If treating 4 or fewer slides, you can use the wash chamber (provided in the kit) filled with 24 mL of Wash Buffer.

Experimental protocols

The following protocols describe how to perform the Click-iT™ EdU Colorimetric IHC Detection assay on FFPE tissue samples.

EdU labeling

10 mg of EdU (Component A) will be sufficient for labeling 1–2 mice depending on the size of the mouse and the treatment method (intraperitoneal injection or drinking water).

In initial experiments, we recommend testing a range of EdU concentrations to determine the optimal concentration. The optimal concentration may vary depending upon the duration of the pulse, with lower concentrations recommended for longer incubations. General recommendations for EdU labeling are listed below.

In vivo labeling—Acceptable EdU incorporation has been observed following injection or media incubation (Table 2).

Table 2. Using EdU in animal species.

Species	Reference*
Nematode (*C. elegans*)	Dorsett M, Westlund B, Schedl T (2009) Genetics 183: 233–247
Flatworm (marine)	BioProbes 61
Cricket	Bando T, Mito T, Maeda Y *et al.* (2009) Development 136: 2235–2245
Mouse	Salic, A (2008) Proc Natl Acad Sci USA 105: 2415–2420 Bonaguidi MA, Peng CY, McGuire T *et al.* (2008) J Neurosci 28: 9194–9204 Kharas MG, Janes MR, Scarfone VM *et al*. (2008) J Clin Ivest 118: 3038–3050 Zeng C *et al*. (2010) Brain Res 1319: 21–32
Rat	Scientific poster, ASCB 2007
Zebrafish larva	BioProbes 57
Zebra finch	Scientific poster ASCB 2007
Human-derived stem cells	McCord AM, Jamal M. Williams ES *et al.* (2009) Clin Cancer Res 15: 5145–5153 Momcilovic O, Choi S, Varum S *et al*. (2009) Stem Cells 27: 1822–1835
*Visit **www.thermofisher.com/edu** for links to PubMed entries, scientific poster, or detailed protocols.	

Deparaffinize tissue sections

2.1 To deparaffinize tissue sections, place the slides in a rack and perform the wash steps listed in Table 3 in a Coplin staining jar or use standard deparaffinization rehydration protocols.

Table 3. Tissue deparaffinization procedure.

Solution	Incubation time
Xylene	5 minutes
Xylene	5 minutes
100% EtOH	5 minutes
100% EtOH	3 minutes
95% EtOH	3 minutes
85% EtOH	3 minutes
75% EtOH	3 minutes
50% EtOH	3 minutes
1X PBS	5 minutes

2.2 To quench endogenous peroxidase enzymes, immerse the slides into a solution of 3% H_2O_2 in PBS for 10 minutes at room temperature.

2.3 Rinse three times in 1X PBS for 2 minutes each.

2.4 Digest the tissue sections with Trypsin-EDTA (Component G) to aid in antigen retrieveal. Optimum digestion time depends on the tissue type. Most other methods of antigen retrieval have been tested and may be used in place of trypsin digestion. DNA unmasking step is not required. See Table 4 for general recommendations.

Note: Covering the tissue with a coverslip during incubation steps will allow the tissue to be covered uniformly. Dry the slide edges prior to adding Trypsin-EDTA to prevent wicking.

Table 4. Recommended Trypsin-EDTA treatment for various tissues.

Species	Tissue type	Incubation time	Temperature
Mouse, embryonic	Cardiac	0–5 minutes	Room temperature
Mouse, adult	Cardiac	20–30 minutes	Room temperature
Rat	Mammary	10 minutes	Room temperature
Rat	Intestine	20–30 minutes	Room temperature
Rat	Uterine	30 minutes	37°C
Zebra fish, adult	Caudal fin	0–5 minutes	Room temperature

2.5 Remove the Trypsin-EDTA solution by washing the tissue sections three times in 1X PBS for 2 minutes each. If using a coverslip, tip the slide to remove the coverslip before proceeding with the wash step.

EdU detection

The following protocol uses 500 µL of Click-iT™ EdU reaction cocktail per tissue section. You can use a smaller volume as long as the remaining reaction components are maintained at the same ratios.

3.1 Prepare a working solution of 1X Click-iT™ EdU Reaction Buffer Additive by diluting the 10X solution (from Step 1.3, page 4) 1:10 in deionized water. Prepare this solution fresh and use it on the same day. Discard any unused 1X solution.

3.2 Prepare the Click-iT™ EdU Reaction cocktail according to Table 5. It is important to add the reaction components in the order listed in the table; otherwise, the reaction will not proceed optimally.

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

Table 5. Click-iT™ EdU Reaction cocktail.

Reaction component*	Number of tissue sections				
	1	2	4	5	10
1X Click-iT™ Reaction buffer (from Step 1.2)	439 µL	878 µL	1.8 mL	2.2 mL	4.4 mL
$CuSO_4$ (Component C)	10 µL	20 µL	40 µL	50 µL	100 µL
Biotin azide (from Step 1.4)	1.2 µL	2.5 µL	5 µL	6 µL	12.5 µL
1X Click-iT™ EdU Reaction Buffer Additive (from Step 3.1)	50 µL	100 µL	200 µL	250 µL	500 µL
Total volume (approximate)	500 µL	1 mL	2 mL	2.5 mL	5 mL
* Add the reaction components in the order listed.					

3.3 Immediately after preparation, add 0.5 mL of the Click-iT™ EdU Reaction cocktail (from Step 3.2) to the prepared tissue sections and allow the solution to spread completely over the surface of the tissue.

3.4 Incubate for 30 minutes at room temperature.

Note: We recommend using a cover slip or a humidified chamber to protect against evaporation.

3.5 *Optional:* Wash the tissue sections with the 1X Click-iT™ EdU Wash Buffer (from Step 1.5, page 4) for 15 minutes. This optional step reduces the background signal.

Note: For this step, we recommend using the Wash chamber included in the kit.

3.6 Wash the tissue sections 3 times for 2 minutes each with 1X PBS at room temperature.

3.7 Add 2 drops of the 1X Streptavidin-Peroxidase Conjugate (Component I) and incubate at room temperature for 30 minutes in a humidified chamber.

Note: Covering the tissue with a coverslip during incubation steps will allow the tissue to be covered uniformly by the reaction components. Dry the slide edges prior to adding the Streptavidin-Peroxidase Conjugate to prevent wicking.

3.8 Remove unbound Streptavidin-Peroxidase Conjugate by washing the tissue sections 3 times for 2 minutes each with 1X PBS at room temperature. If using a coverslip, tip the slide to remove the coverslip before proceeding with the wash step.

3.9 Rinse briefly in deionized water, and remove residual water without allowing the tissue to dry out.

3.10 For each slide to be developed, prepare 200 µL of 1X DAB reaction mixture by combining 10 µL of DAB Chromogen (Component K) with 190 µL of DAB Substrate Buffer (Component J) in a centrifuge tube **immediately before use**. This results in a 1:20 dilution of the DAB Chromogen in DAB Substrate Buffer.

IMPORTANT! Prepare the 1X DAB reaction mixture immediately before use. Discard any unused 1X solution.

Note: Depending on the desired signal strength, you may need to optimize the 1:20 recommended DAB Chromogen dilution. Dilutions of 1:100 to 1:200 for the DAB Chromogen may be needed, if the signal develops too rapidly.

3.11 Add 200 µL of the 1X DAB reaction Mixture (1:20 dilution of DAB Chromogen; from Step 3.10) to each tissue section and incubate at room temperature for 1–10 minutes depending on desired signal intensity. Discard any unused 1X solution.

3.12 Wash each tissue section thoroughly with deionized water and counter-stain, if desired. Mount the tissue sections in standard aqueous or hard mounting medium before imaging. You can also image the tissue sections prior to mounting, if desired.

References

1. Proc Natl Acad Sci USA 105, 2415 (2008); **2.** ChemBioChem 4, 1147 (2003); **3.** J Am Chem Soc 125, 3192 (2003); **4.** Angew Chem Int Ed Engl 41, 2596 (2002); **5.** Angew Chem Int Ed Engl 40, 2004 (2001).

Ordering information

Cat. No.	Product name	Unit size
C10644	Click-iT™ EdU Colorimetric IHC Detection Kit	1 kit
Related products		
14190-144	DPBS, no calcium, no magnesium	500 mL
14190-250	DPBS, no calcium, no magnesium	10 × 500 mL
A10044	EdU (5-ethynyl-2'-deoxyuridine)	50 mg
E10187	EdU (5-ethynyl-2'-deoxyuridine)	500 mg
E10415	EdU (5-ethynyl-2'-deoxyuridine)	5 mg
C10637	Click-iT™ Plus EdU Alexa Fluor™ 488 Imaging Kit *for 50 coverslips*	1 kit
C10638	Click-iT™ Plus EdU Alexa Fluor™ 555 Imaging Kit *for 50 coverslips*	1 kit
C10639	Click-iT™ Plus EdU Alexa Fluor™ 594 Imaging Kit *for 50 coverslips*	1 kit
C10640	Click-iT™ Plus EdU Alexa Fluor™ 647 Imaging Kit *for 50 coverslips*	1 kit
C10289	Click-iT™ AHA Alexa Fluor™ 488 Protein Synthesis HCS Assay	1 kit
C10428	Click-iT™ HPG Alexa Fluor™ 488 Protein Synthesis Assay Kit	1 kit
C10429	Click-iT™ HPG Alexa Fluor™ 594 Protein Synthesis Assay Kit	1 kit
C10327	Click-iT™ RNA Alexa Fluor™ 488 HCS Assay *2-plate size*	1 kit
C10328	Click-iT™ RNA Alexa Fluor™ 594 HCS Assay *2-plate size*	1 kit
C10329	Click-iT™ RNA Alexa Fluor™ 488 Imaging Kit *for 25 coverslips*	1 kit
C10330	Click-iT™ RNA Alexa Fluor™ 594 Imaging Kit *for 25 coverslips*	1 kit
C10350	Click-iT™ EdU Alexa Fluor™ 488 HCS Assay *2-plate size*	1 kit
C10351	Click-iT™ EdU Alexa Fluor™ 488 HCS Assay *10-plate size*	1 kit
C10352	Click-iT™ EdU Alexa Fluor™ 555 HCS Assay *2-plate size*	1 kit
C10353	Click-iT™ EdU Alexa Fluor™ 555 HCS Assay *10-plate size*	1 kit
C10354	Click-iT™ EdU Alexa Fluor™ 594 HCS Assay *2-plate size*	1 kit
C10355	Click-iT™ EdU Alexa Fluor™ 594 HCS Assay *10-plate size*	1 kit
C10356	Click-iT™ EdU Alexa Fluor™ 647 HCS Assay *2-plate size*	1 kit
C10357	Click-iT™ EdU Alexa Fluor™ 647 HCS Assay *10-plate size*	1 kit

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

The information in this guide is subject to change without notice.

For Research Use Only. Not for use in diagnostic procedures.

Revision history: Pub. No. MAN0015678

Revision	Date	Description
C.0	06 November 2016	Corrected the component name in the description of Step 1.3.
B.0	10 January 2017	Corrected the dilution instructions for DAB Chromogen in DAB Substrate Buffer.
A.0	15 March 2016	New document

Manufacturer: Life Technologies Corporation | 29851 Willow Creek Road | Eugene, OR 97402

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