

Optimisation of the Click-iT™ EdU Cell Proliferation Assay for ioOPC-like cells

ioCells™

Optimisation of the Click-iT™ EdU
Cell Proliferation Assay for ioOPC-
like cells

1.0

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1. Introduction

This protocol is based on the manual for the *Click-iT™ EdU Cell Proliferation Kit for Imaging, Alexa Fluor™ 488 dye (ThermoFisher, cat no C10337)*, which was adapted by bit.bio for the quantification of EdU-positive ioOPC-like cells. Please consult ThermoFisher's product manual in the first instance, then refer to bit.bio's recommendations.

Oligodendrocyte progenitor cells (OPCs) have the capacity to proliferate as well as to differentiate into mature oligodendrocytes. As proliferation is a key characteristic of OPCs, we have developed an EdU labelling protocol to determine the proliferation rate of our ioOPC-like cells, which can be expanded and passaged for up to two weeks.

This protocol is intended to provide a quantitative cell proliferation assay for ioOPC-like cells, by quantifying EdU-positive cells during their maintenance phase. The EdU cell proliferation assay is based on Click-iT™ chemistry, a method that uses small reaction moieties instead of antibodies to efficiently conjugate an Alexa Fluor dye to EdU (5-ethynyl-2'-deoxyuridine). As EdU is incorporated in newly synthesised DNA during the cell cycle, proliferating cells can be visualised by fluorescence microscopy. This assay was selected due to its ease of use, compatibility with the immunocytochemistry protocol, its reliability and efficiency in generating data on ioOPC-like cell culture maintenance.

The protocol was optimised for use 48 h after cell seeding and/or passaging and contains minor modifications to the manufacturer's original protocol for cells cultured in multi-well plates rather than coverslips. The assay has been developed in 24-well plates. It is recommended that the imaging method used can capture as much of the well surface as possible in order to obtain a more accurate count of the number of positive cells.



The following protocol recommends general guidelines. We encourage users to optimise the critical steps according to their experimental conditions.

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2. Materials and equipment

2.1. Cells

- ioOPC-like cells (io1100)

2.2. Reagents and equipment for cell culture

For a complete list of equipment and reagents needed for ioOPC-like cells culture, consult the [ioOPC-like cells User Manual](#).

2.3. Application-specific reagents and equipment

Table 1 Reagents specific for the EdU cell proliferation assay.

Reagent	Supplier	Cat no	Storage
Click-iT™ EdU Cell Proliferation Kit for Imaging, Alexa Fluor™ 488 dye	ThermoFisher	C10337	4°C
DPBS	ThermoFisher	14190169	Room temperature
Deionised water	Sigma-Aldrich	38796-1L	Room temperature
DAPI	Bio-Techne	5748/10	-20°C

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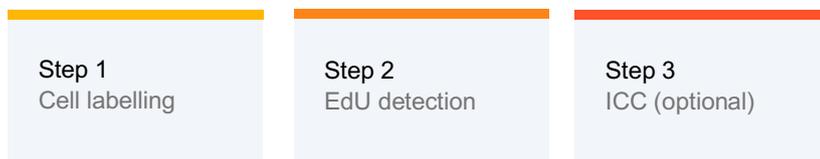
3. Protocol

This protocol is split into 3 main steps:

Step 1: Cell labelling

Step 2: EdU detection

Step 3: Immunocytochemistry (ICC) (optional)



Before starting:

- Cells should be thawed, seeded and cultured as indicated in the [ioOPC-like cells User Manual](#).
- EdU labelling should be performed 48 h after cell thawing and/or after passaging following the protocol below.
- This protocol has been optimised for 24 well plates – if the assay is required to be conducted in other well formats, please adjust reagent volumes to half working volume of the wells.
- The commercial kit contains 7 components (labelled A to G), listed in Table 2. Please consult the manufacturer's manual for a description of each component and storage conditions after reconstitution.



For support regarding cell culture of ioOPC-like cells, please refer to the User Manual. If you need assistance, visit www.bit.bio/support-hub.

Table 2 Click-iT kit components and recommended preparation and storage conditions.

Component	Component ID	Preparation of stock solutions	Storage after reconstitution
EdU	A	10 mM Prepare a 10 mM EdU Stock Solution by adding 2 mL of DMSO	-20°C
Alexa Fluor® azide	B	Prepare Alexa Fluor® azide Solution by adding 70 µL of DMSO	-20°C
Dimethylsulfoxide (DMSO)	C	-	-
Click-iT® EdU reaction buffer	D	1x Prepare a 1X solution by adding 36 mL of deionised water to the 4 mL buffer	-20°C
CuSO ₄	E	-	-
Click-iT® EdU buffer additive	F	10X Prepare a 10X solution by adding 2 mL of deionised water	-20°C
Hoechst 33342	G	-	-

3.1. Cell labelling

- 3.1.1 Prepare OPC- maintenance medium as per the [ioOPC-like cells User Manual](#) and allow to reach room temperature.
- 3.1.2 Prepare a 10 mM EdU stock as per table 2
- 3.1.3 Prepare sufficient volume of OPC-maintenance medium with EdU component as per table 3
- 3.1.4 Perform a 50% medium change with the OPC-maintenance medium with EdU component.
- 3.1.5 Incubate cells at 37°C for 24 h.
- 3.1.6 Following incubation, remove medium and fix cells using 250µL of 4% PFA for 10 minutes at RT according to the immunocytochemistry protocol for ioOPC-like cells.

Table 3 Preparation of OPC maintenance medium with EdU component.

Reagent/ Media	For 1 mL	For 10 mL
OPC-maintenance medium	1 mL	10 mL
EdU stock solution (10 mM)	2 µL	20 µL

3.2. EdU detection

- 3.2.1 Prepare 1X Click-iT EdU reaction buffer as per table 2
- 3.2.2 Prepare 10X Click-iT EdU buffer additive as per table 2
- 3.2.3 Prepare 1X Click-iT EdU buffer additive by diluting the 10X stock 1:10 in [deionised](#) water.
- 3.2.4 Prepare Alexa Fluor azide as per table 2.
- 3.2.5 Prepare the Click-iT EdU reaction cocktail according to Table 4 below.

Table 4 – Click-iT EdU reaction cocktail components and volumes.

Reaction components	Number of wells per 24-well plate				
	1	2	4	5	10
1X Click-iT EdU reaction buffer	215 µL	430 µL	860 µL	1075 µL	2.2 mL
CuSO ₄ (Component E)	10 µL	20 µL	40 µL	50 µL	100 µL
Alexa Fluor azide	0.6 µL	1.2 µL	2.5 µL	3 µL	6 µL
1X Click-iT EdU buffer additive	25 µL	50 µL	100 µL	125 µL	250 µL
Total volume	0.25 mL	0.5 mL	1 mL	1.25 mL	2.55 mL



When making the Click-iT EdU reaction cocktail, Components should be added in order listed in the table

- 3.2.6 Permeabilise and block the cells as per immunocytochemistry protocol for ioOPC-like cells (section 3.2. Permeabilisation and blocking).
- 3.2.7 Wash the cells twice with 250 µL DPBS.
- 3.2.8 Add 250 µL of Click-iT EdU reaction cocktail per well. Incubate for 30 min at RT in the dark by wrapping the plate in aluminium foil.
- 3.2.9 Wash the cells twice with 250 µL DPBS.

3.2.10 If not proceeding to section 3.3, add a nuclear stain such as DAPI (2 µg/mL) for 5 min at RT or Hoechst 33342 as per the manufacturer's protocol.

3.2.11 Wash the cells twice with 250 µL DPBS.

3.2.12 **EdU visualisation:** Signal can be visualised using fluorescent microscopy, if using the Alexa Fluor 488 kit a microscope capable of detecting FITC will be needed. The number of EdU positive nuclei can be quantified if desired by imaging multiple sites in the well using a system such as ImageXpress. An example of expected results can be found in Figure 1 below.

3.3. Immunocytochemistry staining (optional)

Following EdU detection (section 3.2.), the cells can be further stained for additional markers. If this is the case, follow the steps in the [ioOPC-like cells immunocytochemistry protocol](#), starting with section 3.3 Primary antibody labelling. It is recommended that both protocols are read in advance so that any modifications to the assay can be implemented beforehand.

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4. Reference data

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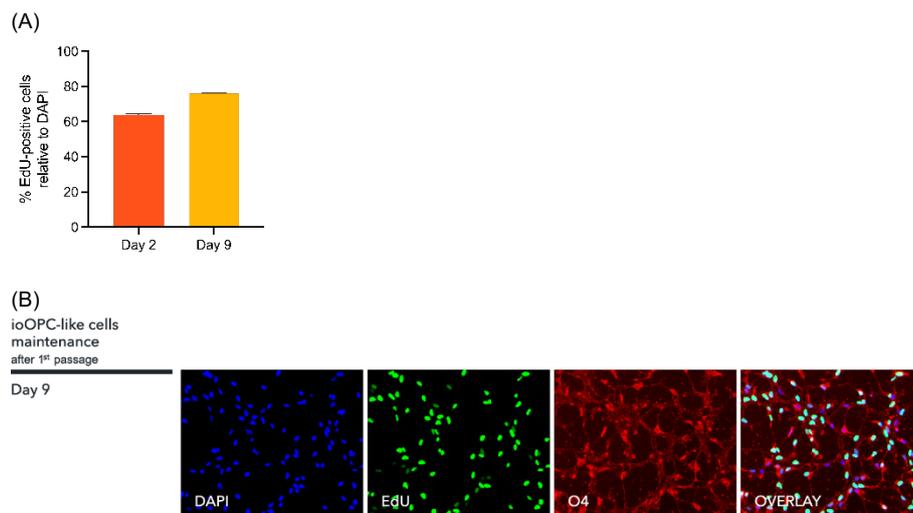


Figure 1 Treatment of ioOPC-like cells with Click-iT EdU cell proliferation assay. (A) EdU quantification in ioOPC-like cells at day 2 and day 9 (2 days after the first passage). Each bar represents the mean \pm SEM of 32 image sites acquired across 2 wells of a 24-well plate. (B) Immunostaining imaging of ioOPC-like cells after treatment of Click-iT EdU at day 9 (48 hours after 1st passage). DAPI (blue) EdU (488, green), O4 (467, red).

5. Troubleshooting

Issue	Possible causes	Solutions
Low signal	EdU reaction cocktail was not used fresh	Ensure the EdU reaction cocktail is used immediately after preparing
	Insufficient fixation and/or permeabilisation of the cells	Fix and permeabilise the cells as described in the ioOPC-like cells immunocytochemistry protocol
	Buffers contain metal ions or metal chelators	Make sure to use <u>deionised</u> water when preparing buffers

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6. Related protocols and resources

- [ioOPC-like cells User Manual](#)
- [Immunocytochemistry Protocol for ioOPC-like cells and ioOligodendrocyte-like cells](#)

For the complete list of protocols and resources from bit.bio, please refer to <https://www.bit.bio/resources>.