molecular probes®
by life technologies™

Click-iT® Plus OPP Protein Synthesis Assay Kits

Catalog nos. C10456, C10457, C10458, C10459

Table 1 Contents and storage

Material	C10456	C10457	C10458	Concentration	C10459*	Storage**
Click-iT® OPP Reagent (Component A)	30 µL	30 µL	30 µL	20 mM in DMSO	5 mg	• 2°C–8°C • Desiccate • Protect from light • DO NOT FREEZE
Alexa Fluor® picolyl azide (Component B)	1 vial of Alexa Fluor® 488 picolyl azide (70 uL)	1 vial of Alexa Fluor® 594 picolyl azide (70 uL)	1 vial of Alexa Fluor® 647 picolyl azide (70 uL)	DMSO solution	NA	
Click-iT® OPP Reaction Buffer (Component C)	4 mL	4 mL	4 mL	10X solution containing Tris-buffered saline	NA	
Copper Protectant (Component D)	1 vial, 600 uL	1 vial, 600 uL	1 vial, 600 uL	NA	NA	
Click-iT® Reaction Buffer Additive (Component E)	400 mg	400 mg	400 mg	NA	NA	
Click-iT® Reaction Rise Buffer (Component F)	25 mL	25 mL	25 mL	Contains 2 mM sodium azide	NA	
NuclearMask™ Blue Stain (Component G)	25 µL	25 µL	25 µL	2000X concentrate in water	NA	

* Cat. no. C10459 includes only the Click-iT® OPP Reagent supplied in a larger quantity for use in applications where additional OPP material is required (i.,e. whole animal imaging). The Click-iT® Plus OPP Alexa Fluor® Protein Synthesis kit provides the remaining reagents needed for detection.

**These storage conditions are appropriate when storing the entire kit upon receipt. For optimal storage conditions for each component, see vial labels. When stored as directed, the product is stable up to 1 year after receipt.

NA = Not applicable.

Number of assays: Sufficient material is supplied for 25 coverslips or 250 wells using 96-well plates, based on the protocol described here. For larger quantities, inquire at **www.lifetechnologies.com**.

Approximate fluorescence excitation and emission maxima, in nm: Alexa Fluor® 488 picolyl azide: 495/519; Alexa Fluor® 594 picolyl azide: 590/615; Alexa Fluor® 647 picolyl azide: 650/670; NuclearMask™ Blue Stain: 350/451.

Introduction

The ability to detect and characterize newly synthesized proteins, changes in spatial or temporal protein expression patterns, or protein degradation resulting from disease, drug treatments, or environmental changes, is an important parameter in cytotoxicity measurements. The Click-iT® Plus OPP Protein Synthesis Assays provide a fast, sensitive, non toxic, specific, and non-radioactive method for the detection of nascent protein synthesis[1] utilizing fluorescence microscopy and high-throughput imaging (high content screening, HCS).

Changes in protein expression are detected by the addition of Click-iT® OPP (O-propargyl-puromycin) to actively growing cells. The Click-iT® OPP reagent is a puromycin analog containing an alkyne moiety. When added to culture media, OPP is readily taken up by actively growing cells. OPP inhibits protein synthesis by disrupting peptide transfer on ribosomes causing premature chain termination during translation. Addition of the Alexa Fluor® picolyl azide and the Click reaction reagents leads to a chemoselective ligation or "click" reaction between the picolyl azide dye and the alkyne OPP, allowing the modified proteins to be detected by imaged-based analysis.

Unlike traditional methods, such as ^{35}S-Methionine, OPP is not an amino acid analog; thus, OPP can be added directly to cells in complete media (i.e., methionine-containing) or used to detect in vivo protein synthesis. Because OPP can be used in complete cell culture media it can be used with cell lines that are sensitive to media exchanges or incubation in methionine-free media.

The click reaction integral to the Click-iT® Plus OPP uses bioorthogonal (biologically unique) moieties (picolyl azide and alkyne moieties) to fluorescently label nascently translated polypeptides, producing low backgrounds and high detection sensitivities. Click-iT® Plus OPP Alexa Fluor® Protein Synthesis Assays have been successfully tested in a number of cell types including NIH 3T3, BPAE, U-2 OS, CHO-M1, HeLa and A549 cells with reagents that inhibit protein synthesis including the translational elongation inhibitor cycloheximide (Figures 1A and 1B, page 3). In addition, drugs that inhibit protein clearance, such as MG134, show a dose-dependent inhibition of clearance (Figure 2, page 3).

The mild reaction conditions for the Click-iT® Plus OPP assays have been demonstrated to preserve cell morphology, the binding properties of phalloidin, and the signal from fluorescent proteins such as GFP. The Click-iT® Plus OPP Alexa Fluor® Protein Synthesis Assay kits can be multiplexed with fluorescently-labeled phalloidin and GFP-based fluorescent reporters (Figure 3, page 4).The kit contains all of the components needed to label and detect the incorporated OPP into newly translated proteins on samples from adherent cells.

Figure 1 Using Click-iT® Plus OPP to monitor the inhibition of the protein synthesis. The dose-dependent decrease was monitored using either the Alexa Fluor® 488 (Figure 1A) or the Alexa Fluor® 594 (Figure 1B) version of the Click-iT® Plus OPP Protein Synthesis Assay Kit.

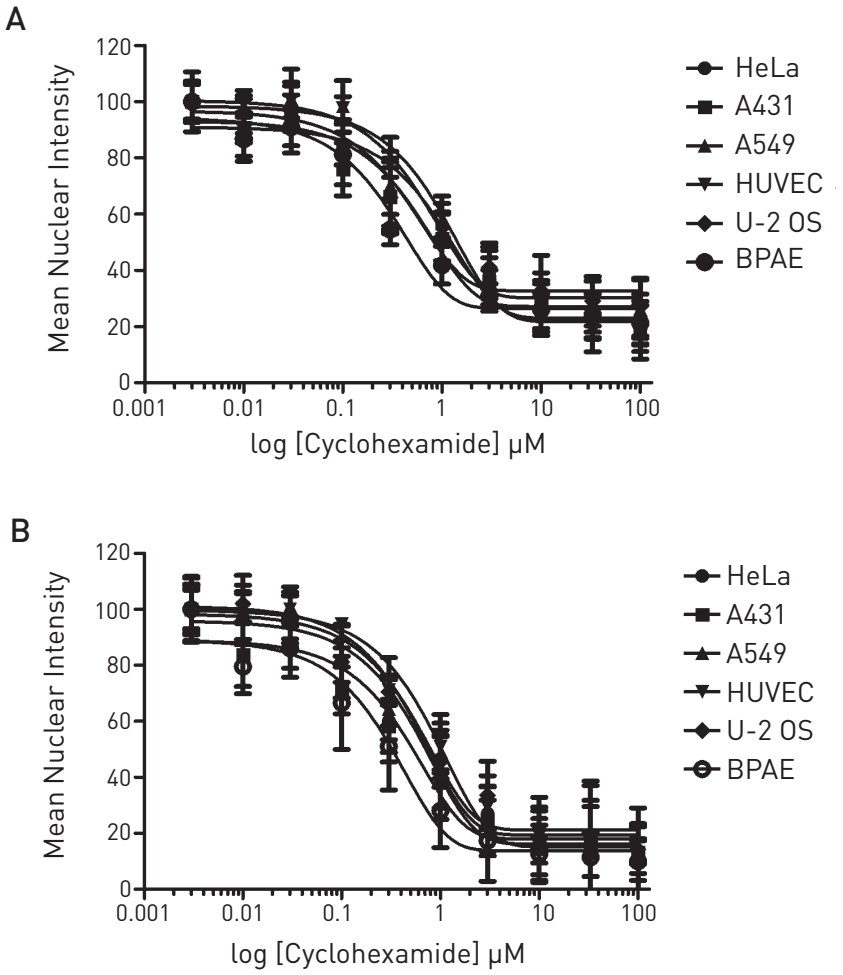


Figure 2 Inhibition of the proteasome leads to a dose-dependent increase the Click-iT® Plus OPP signal. Blockade of protein clearance via the proteasome with MG132 causes a dose-dependent accumulation of OPP-labeled proteins in HeLa cells.

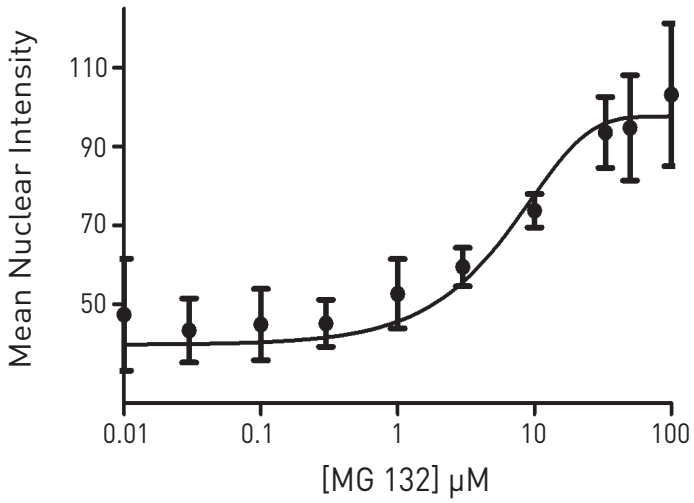
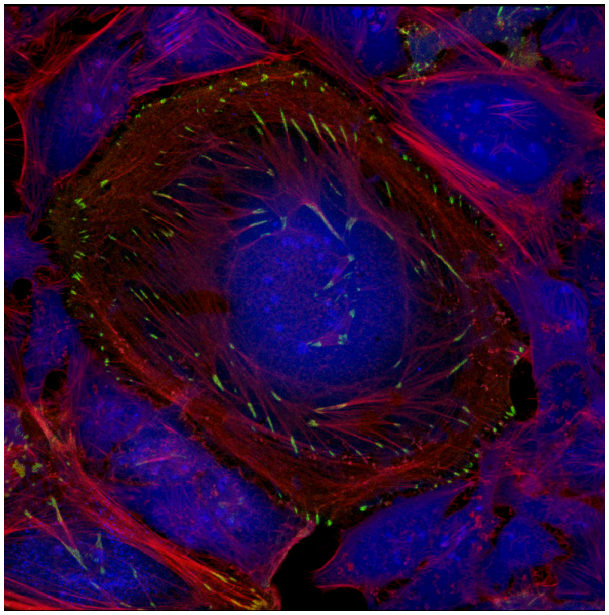


Figure 3 Multiplexing using Click-iT® Plus OPP Alexa Fluor® 647, Alexa Fluor® 568 conjugated phalloidin, and GFP. Because of the mild Click-iT® Plus reaction conditions, fluorescent signals from GFP and Alexa Fluor® 568 conjugated phalloidin can be multiplexed with the Alexa Fluor® - OPP signal.



Before you begin

Materials required but not provided

- 96-well plates or culture dishes designated for fluorescent image analysis (as recommended for the specific imaging instrument)
- Phosphate buffered saline (PBS, pH 7.2–7.6)
- Fixative (i.e., 3.7% Formaldehyde in PBS)
- Permeabilization reagent (i.e., 0.5% Triton® X-100 in PBS)
- Deionized water

Cautions

- NuclearMask™ Blue Stain (Component G) is a known mutagen. Use the dye with appropriate precautions.
- DMSO (in Components A and B), is known to facilitate the entry of organic molecules into tissues. 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. Always wear protective laboratory clothing and gloves when handling reagents containing DMSO.

Prepare reagents

1.1 Allow vials to completely thaw and warm to room temperature before opening.

1.2 Prior to use, briefly centrifuge Click-iT® OPP Reagent (Component A) and NuclearMask™ Blue Stain (Component G) to maximize reagent recovery.

1.3 To prepare a 10X stock solution of the Click-iT® Reaction Buffer Additive (Component E), add 2 mL deionized water to the vial and mix until completely dissolved. After use, store any remaining stock solution at ≤–20°C.

Note: When stored as directed, this stock solution is stable for up to 1 year.

1.4 Prepare 40 mL of 1X Click-iT® OPP Reaction Buffer by transferring all of the solution in the Component C bottle (4 mL) to 36 mL of deionized water. Rinse the Component C bottle with some of the diluted Click-iT® OPP Reaction Buffer to ensure the transfer of all of the 10X concentrate.

Note: To prepare smaller amounts of 1X Click-iT® OPP Reaction Buffer, dilute an aliquot from the Component C bottle 1:10 with deionized water. After use, store any remaining 1X solution at 2°C–8°C. When stored as directed, 1X Click-iT® OPP Reaction Buffer is stable for 6 months.

Experimental Protocols

Label cells with OPP

The following protocols were developed using a number of cell types including NIH 3T3, BPAS, U-2 OS, CHO-M1, HeLa, and A549. The amount of Click-iT® OPP Reagent (Component A) was optimized, depending on cell-type specific media, to a concentration between 2–20 µM. The following protocols can be adapted for any adherent cell type. For initial experiments, we recommend testing a range of Click-iT® OPP Reagent concentrations to determine the optimal concentration for your cell type and experimental conditions. Growth medium, cell density, cell type variations, and other factors may influence labeling. Although sufficient material is included for standard dose response studies, additional OPP (Cat. no. C10459) is available.

Method 1: Drug pre-incubation

2.1 Plate cells at the desired density and allow cells to recover overnight before additional treatment.

2.2 Treat the cells with the drug of your choice.

2.3 Dilute Click-iT® OPP (Component A) 1:1000 in cell culture medium to prepare a 20 µM final working solution.

2.4 Remove the drug-containing medium (step 2.2) and add 1 mL per coverslip or 100 µL per well of medium with 20 µM Click-iT® OPP working solution (prepared in step 2.3).

2.5 Incubate cells for 30 minutes under conditions optimal for the cell type.

2.6 Proceed to **Fix and permeabilize** (page 6) followed by **Click-iT® OPP detection** (page 6).

Method 2: Click-iT® OPP and drug co-incubation

3.1 Plate cells at the desired density and allow cells to recover overnight before additional treatment.

3.2 Dilute Click-iT® OPP (Component A) 1:1000 in pre-warmed cell culture medium to prepare a 20 µM working stock solution.

3.3 Add the drug of your choice to the working stock solution of Click-iT® OPP (prepared in step 3.2).

3.4 Remove medium from the cells and add 1 mL per coverslip or 100 µL per well of medium with 20 µM Click-iT® OPP and the drug (prepared in step 3.3).

3.5 Incubate for 30 minutes under conditions optimal for your cell type.

3.6 Proceed to **Fix and permeabilize** followed by **Click-iT® OPP detection**.

Fix and permeabilize

The following protocol is optimized with a fixation step using 3.7% formaldehyde in PBS followed by a permeabilization step using 0.5% Triton® X-100, but is amenable to other fixation/permeabilization reagents such as ethanol and methanol.

4.1 After incubation, remove the medium containing Click-iT® OPP and wash the cells once with PBS. Remove PBS.

4.2 Add 1 mL per coverslip or 100 µL per well of 3.7% formaldehyde in PBS. Incubate for 15 minutes at room temperature. Remove fixative.

4.3 Add 1 mL per coverslip or 100 µL per well of 0.5% Triton® X-100 in PBS and incubate for 15 minutes at room temperature.

Click-iT® OPP detection

5.1 Prepare 1X Click-iT® OPP Reaction Buffer Additive by diluting the 10X solution (prepared in step 1.3) 1:10 in deionized water. Prepare this solution fresh and use the solution on the same day.

5.2 Prepare Click-iT® Plus OPP reaction cocktail according to Table 2.

Table 2 Click-iT® Plus reaction cocktail

Reaction components	Number of coverslips/Number of wells of a 96-well plate			
	1 coverslip/ 10 wells	5 coverslips/ 50 wells	10 coverslips/ 100 wells	20 coverslips/ 200 wells
Click-iT® OPP Reaction Buffer (1X concentrate, prepared in step 1.4)	880 µL	4.4 mL	8.8 mL	17.6 mL
Copper Protectant (Component D)	20 µL	100 µL	200 µL	400 µL
Alexa Fluor® picolyl azide (Component B)	2.5 µL	12.5 µL	25 µL	50 µL
Click-iT® Reaction Buffer Additive (1X solution, prepared in step 5.1)	100 µL	500 µL	1 mL	2 mL
Total reaction volume	**1 mL**	**5 mL**	**10 mL**	**20 mL**

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

5.3 Remove the permeabilization buffer (step 4.3) and wash cells twice with 1 mL per coverslip or 100 µL per well of PBS. Remove the wash solution.

5.4 Add 1 mL per coverslip or 100 µL per well of Click-iT® Plus OPP reaction cocktail (prepared in step 5.2) to each well and mix well.

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

5.6 Remove the reaction cocktail and wash once with 1 mL per coverslip or 100 µL per well of Click-iT® Reaction Rinse Buffer (Component F). Remove the rinse buffer.

DNA staining

The following protocol is based on 100 µL of HCS NuclearMask™ Blue Stain working solution per well.

6.1 Dilute HCS NuclearMask™ Blue Stain (Component G) solution 1:2000 in PBS to obtain a 1X HCS NuclearMask™ Blue Stain working solution.

6.2 Remove any wash solution from the cells.

6.3 Add 1 mL per coverslip or 100 µL per well of 1X HCS NuclearMask™ Blue Stain working solution (prepared in step 6.1). Incubate for 30 minutes at room temperature, protected from light.

6.4 Remove the HCS NuclearMask™ Blue Stain solution and wash twice with PBS. Remove the wash solution and proceed to **Imaging and analysis**.

Imaging and analysis

7.1 Add PBS to each well. Seal the plate with plate sealing film, if desired.

7.2 Scan the plate using automated imaging platform with appropriate filters: FITC for Alexa Fluor® 488, Texas Red® for Alexa Fluor® 594, and Cy®5 for Alexa Fluor® 647.

Note: Nascent protein synthesis is assessed by determining signal intensity in the fluorescent channel in the ring around or within the nucleus as defined by NuclearMask™ Blue Stain.

References

1. PNAS, 109, 913 (2012).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
C10456	Click-iT® Plus OPP Protein Synthesis Assay Kit *Alexa Fluor® 488 picolyl azide *	1 kit
C10457	Click-iT® Plus OPP Protein Synthesis Assay Kit *Alexa Fluor® 594 picolyl azide*	1 kit
C10458	Click-iT® Plus OPP Protein Synthesis Assay Kit *Alexa Fluor® 647 picolyl azide *	1 kit
C10459	Click-iT® Plus OPP Reagent	5 mg

Related Products

C10102	Click-iT® AHA (L-Azidohomalanine)	5 mg
C10289	Click-iT® AHA Alexa Fluor® 488 Protein Synthesis HCS Assay	2 plates
C10186	Click-iT® L-Homopropargylglycine (HPG)	5 mg
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
C10329	Click-iT® RNA Alexa Fluor® 488 Imaging Kit	1 kit
C10330	Click-iT® RNA Alexa Fluor® 594 Imaging Kit	1 kit
C10327	Click-iT® RNA Alexa Fluor® 488 HCS Assay	1 kit
C10328	Click-iT® RNA Alexa Fluor® 594 HCS Assay	1 kit

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6 February 2014

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