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Click-&-Go® Plus OPP Protein Synthesis Assay Kit

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Cat. No. **CCT-1492, 1493, 1494, 1495, 1496**

Introduction

Although protein synthesis is a conserved and essential cellular function, it is often regulated in a cell-type-specific manner to influence cell fate, growth and homeostasis. Most methods used to measure protein synthesis depend on metabolically labeling large numbers of cells with radiolabeled amino acids, stable isotope-labeled amino acids, bioorthogonal noncanonical amino acid tagging (L-azidohomoalanine or homopropargylglycine or their combination). Because these methods typically depend on specialized growth conditions, they have been largely restricted to yeast, bacteria and cell lines. Application of these techniques for investigating protein synthesis within mammalian systems *in vivo* has been challenging. The use of O-propargyl-puromycin (OPP), an analog of puromycin that contains a terminal alkyne group, has facilitated the quantification of protein synthesis within individual cells *in vivo*. OPP enters the acceptor site of ribosomes and incorporates into nascent polypeptide chains. Unlike traditional methods mentioned above, 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. It also can be used with cell lines that are sensitive to media exchanges or incubation in methionine-free media. The combination of high cell permeability and signal-to-noise ratio makes OPP an ideal candidate compound to study nascent proteomes across a wide array of cellular types and conditions. The kit contains all of the components needed to detect incorporated OPP with fluorescent AZDye Azide Plus (Alexa Fluor® equivalent), and blue-fluorescent Hoechst 33342 dye for nuclear staining. A sufficient amount of reagents is provided for imaging 25 coverslips or 250 wells using 96-well plates.

Kit Contents

Component	Concentration	Amount
OPP Reagent (Component A)	n/a	0.5 mg
AZDye Azide Plus (Component B)	n/a	1 vial
Reaction Buffer (Component C)	10X solution	3 mL
Copper Catalyst (Component D)	100X solution	0.5 mL
Reducing Agent (Component E)	n/a	400 mg
Wash Buffer (Component F)	n/a	25 mL
Hoechst 33342 (Component G)	10 mg/mL	50 µL

Materials required but not provided

- Fixative (for example, 3.7% Formaldehyde in PBS)
- Permeabilization reagent (for example, 0.5% solution of Triton® X-100 in PBS)
- Coverslips/microscope slides, mounting media
- PBS buffer, pH 7.4
- Deionized water

Additional information

Hoechst 33342 (Component G) is a known mutagen. Use the dye with appropriate precautions.

Wash Buffer (Component F) contains 2 mM sodium azide.

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Material Preparation

OPP Reagent (Component A)	Add 50 µL of DMSO to make 20 mM stock solution. Store at -20°C. This stock solution is stable for up to 1 year.
AZDye Azide Plus (Component B)	Add 150 µL of deionized water or DMSO. Protect from light. Store at -20°C. This stock solution is stable for up to 6 months.
Reaction Buffer (Component C)	To prepare required amount of **1x reaction buffer** (see Table 1), dilute the appropriate volume from **Reaction Buffer (Component C)** bottle 1:10 with deionized water. To convert the entire amount of **10x Reaction buffer** into 1x working solution, transfer entire bottle of **10x Reaction Buffer** (3 mL) into 27 mL of deionized water. Store undiluted 10X reaction buffer at 2–4°C. The 10X solution is stable for 1 year.
Copper Catalyst (Component D)	Ready to use. When stored as directed, this stock solution is stable for up to 1 year.
Reducing Agent (Component E)	Prepare enough of fresh **1x solution** of **Reducing Agent (Component E)** for one day. Weigh our 20 mg of **Reducing Agent (Component E)** into 2 mL vial, add 1.8 mL of deionized water. Vortex until completely dissolved.
Wash Buffer (Component F)	Ready to use. Store at 4°C. When stored as directed, this stock solution is stable for up to 1 year.
Hoechst 33342 (Component G)	Ready to use. Store at -20°C. When stored as directed, this stock solution is stable for up to 1 year.

Labeling cells with OPP

The following protocols were developed using a number of cell types and can be adapted for any types of adherent cell lines. The concentration of OPP reagent is cell-type and media specific and usually between 2-30 µM. For the initial experiment we recommend using 20 µM concentration of OPP reagent, however investigators may wish to determine the optimal concentration and experimental conditions for each cell type on a small-scale first. Cell type variations, growth medium and cell density may influence labeling efficiency.

1.1 Seed the cells at desired density 24 hours prior to the experiment.

1.2 Treat the cells with the drug of your choice or perform OPP and your drug co-incubation.

1.3 Dilute OPP reagent (Component A) at appropriate concentration in cell culture medium (for example 1:1,000 to get 20 µM working solution).

1.4 Incubate the cells for optimal time (usually 30 minutes).

1.5 Proceed to cell fixation and permeabilization.

Cell fixation and permeabilization

The following protocol is provided for the fixation step using 3.7% formaldehyde in PBS followed by a 0.5% Triton®X-100 permeabilization step. Protocols using other fixation/permeabilization reagents, such as methanol and saponin also can be used.

2.1 Transfer each coverslip into a single well. For convenient processing use 6-well plates.

2.2 After OPP labeling, remove the media and wash coverslips once with PBS, then remove PBS.

2.3 Add 1 mL of 3.7% formaldehyde in PBS to each well containing the coverslips. Incubate for 15 minutes at room temperature.

2.4 Remove the fixative and wash the cells once with PBS.

2.5 Remove PBS. Add 1 mL of 0.5% Triton® X-100 in PBS to each well, then incubate at room temperature for 15 minutes.

OPP detection

Note: 1 mL of the reaction cocktail is used per coverslip. A smaller volume can be used as long as the remaining reaction components are maintained at the same ratios.

3.1 Weigh our 20 mg of **Reducing Agent (Component E)** into 2 mL vial, add 1.8 mL of deionized water, vortex until completely dissolved. This solution should be freshly prepared and used on the same day.

3.2 Prepare required amount of the reaction cocktail according to Table 1. **Add the ingredients in the order listed in the table.** Use the reaction cocktail within 5 minutes of preparation.

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Table 1.

Reaction component	Number of coverslips or wells of a 96-well plate			
	1 coverslip or 10 wells	5 coverslips or 50 wells	10 coverslip or 100 wells	20 coverslip or 200 wells
1x Reaction Buffer **(Material preparation)**	885 µL	4.4 mL	8.8 mL	17.7 mL
Copper Catalyst **(Component D)**	10 µL	50 µL	100 µL	200 µL
AZDye Azide Plus Solution **(Material preparation)**	5 µL	25 µL	50 µL	100 µL
1x Reducing Agent **(step 3.1)**	100 µL	500 µL	1 mL	2 mL
Total Volume	1 mL	5 mL	10 mL	20 mL

3.3 Remove the permeabilization buffer (step 1.4). Wash the cells in each well twice with 1 mL of PBS. Remove PBS.

3.4 Immediately add 1 mL of the **Reaction Cocktail** to the sample. Evenly distribute the reaction cocktail over the sample.

3.5 **Protect from light,** and incubate the plate for 20 minutes at room temperature.

3.6 Remove the reaction cocktail. Wash each well once with 1 mL of Wash Buffer. Remove the Wash Buffer.

3.7 Wash each well with 1 mL of PBS. Remove PBS.

At this point the samples are ready for **DNA staining.** If no **DNA staining** is desired, proceed to **Imaging.**

If antibody labeling of the samples is desired, proceed to labeling according to manufacturer's recommendations. **Keep the samples protected from light during incubation.**

DNA staining

4.1 Prepare 1x **Hoechst 33342 solution** by diluting stock solution of **Hoechst 33342 (Component G)** 1:2,000. The final concentration of 1x **Hoechst 33342 solution** is 5 µg/mL.

Final concentrations of 1x **Hoechst 33342** may range from 2 µg/mL to 10 µg/mL.

4.2 Add 1 mL of 1x **Hoechst 33342** solution per well. **Protect from light.** Incubate for 15 minutes at room temperature.

4.3 Remove the Hoechst 33342 solution.

4.4 Wash each well twice with 1 mL of PBS.

4.5 Remove PBS.

4.6 For coverslips, mount them on the slides according to the mounting media manufacturer's protocol. For wells, add another portion of PBS to each well and proceed to scanning.

Imaging

Labeled cells are compatible with all methods for slide preparation. See **Table 2** for approximate fluorescence excitation/emission maxima for AZDyes and Hoechst 33342 dye bound to DNA.

Table 2.

	Excitation (nm)	Emission (nm)
AZDye 405 Azide Plus	402	461
AZDye 488 Azide Plus	495	519
AZDye 555 Azide Plus	550	570
AZDye 594 Azide Plus	590	617
AZDye 647 Azide Plus	650	670
Hoechst 33342 bound to DNA	350	461

Selected References

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2. Forester, C.M., *et al.* (2018). Revealing nascent proteomics in signaling pathways and cell differentiation. *PNAS.* **115:** 2353-8.
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