Version 5a, Last updated 8 December 2025

ab239725
Protein Synthesis Assay Kit

For the detection of nascent proteins in suspension or adherent cell cultures.

This product is for research use only and is not intended for diagnostic use.

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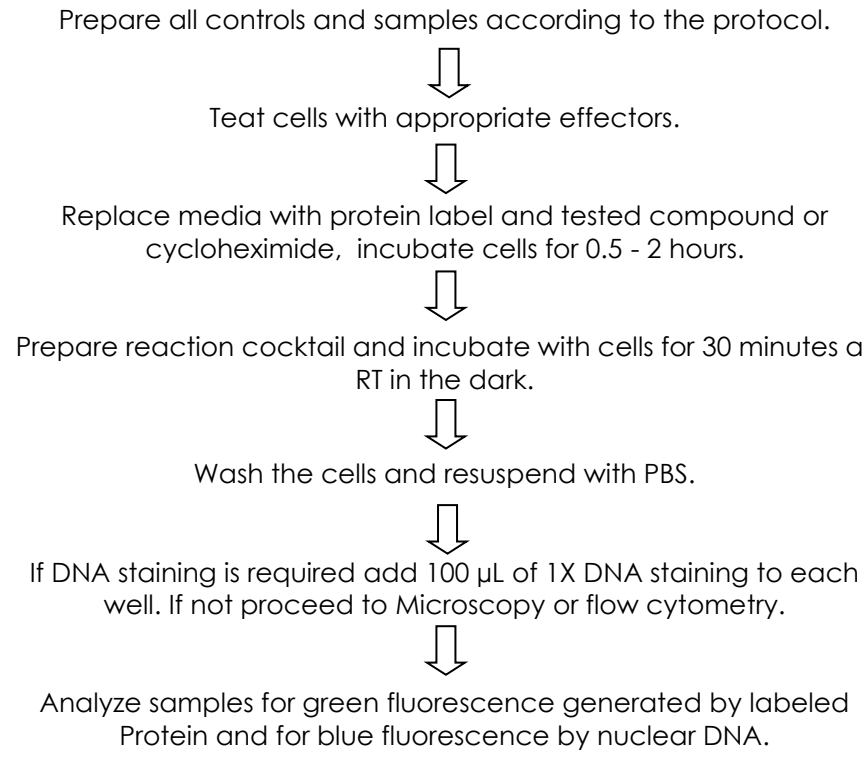
1. Overview

The Protein Synthesis Assay Kit (ab239725) utilizes a novel and robust chemical method based on an alkyne containing and cell permeable analog of puromycin, O-Propargyl-puromycin (OP-puro). Once inside the cell, OP-puro stops translation by forming covalent conjugates with nascent polypeptide chains.

Truncated polypeptides are rapidly turned over by the proteasome and can be detected based on a click reaction with the fluorescent azide. Unlike methionine analogs, OP-puro does not require methionine-free conditions and can be used to label nascent proteins directly in the cell culture.

2. Protocol Summary



3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components after preperatiion. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition after preparation
10X Wash Buffer IV	25 mL	4°C
Fixative Solution I	10 mL	-20°C
10X Permeabilization Buffer	25 mL	4°C
400X Protein Label	25 µL	-20°C
100X Copper Reagent	100 µL	-20°C
Fluorescent Azide II	100 µL	-20°C
Reducing Agent	1 vial	-20°C
1000X DAPI	20 µL	-20°C
100X Cycloheximide	10 µL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Tissue culture vessels and appropriate culturing media.
- Phosphate Buffered Saline (PBS, pH 7.4).
- Sterile 0.1% Gelatin Solution (optional, only required for suspension cells).
- Flow cytometer equipped with laser capable of excitation at 488 nm wavelength (FL-1).
- Fluorescence microscope capable of excitation and emission at 440/490 and UV filter.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 10X Wash Buffer IV:

Thaw at 37°C to dissolve completely. Dilute the 10X stocks 1:10 in sterile water, mix well.

6.2 Fixative Solution I:

Ready to use as supplied. Divide into working aliquots and store at -20°C protected from light.

6.3 10X Permeabilization buffer:

Thaw at 37°C to dissolve completely. Dilute the 10X stocks 1:10 in sterile water, mix well.

6.4 400X Protein label:

Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.5 100X Copper Reagent:

Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.6 Fluorescent Azide II:

Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.7 20X Reducing Agent:

Resuspend the reducing agent with 560 µL Type I water. Mix well and store solution at -20°C protected from light. While in use, keep on ice and minimize light exposure.

Δ Note: For older lots (20X Reducing Agent 500 µL), ready to use as provided. Store solution at -20°C protected from light.

6.8 1000X DAPI:

Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.9 100X Cyclohexamide:

Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

7. Assay Procedure

Δ Note: This assay was developed with HeLa (adherent) and Jurkat (suspension) cells and can be modified for any cell line. The protocol below refers to a 96-well tissue culture plate; adjust volumes accordingly for other plate formats. The assay volume is 100 µL. Growth conditions, cell number per well and other factors may affect the incorporation rate of the Protein Label; therefore optimize the assay for your cell type.

Δ Note: We suggest an initial test of several Protein Label concentrations to find best conditions for your experimental design. Avoid stressing the cells by washes or temperature changes prior to incubation with Protein Label.

Δ Note: All steps should be carried out at room temperature (RT) unless otherwise specified; equilibrated all buffers to RT prior to the experiment.

7.1 Labeling of control and experimental cells; method with drug pre-incubation:

7.1.1 Obtain cell suspension of desired density and seed directly into tissue culture vessels, or on coverslips for high resolution microscopy.

7.1.2 **To immobilize suspension cells for microscopy**: add 100 µL of 0.1% gelatin solution directly into the wells. Tilt the plate to cover the entire well surface and place it in a tissue culture hood for 1 hour.

7.1.3 Gently remove the gelatin solution and seed your cells. Allow the cells to recover overnight before the treatment. Next day, treat the cells with appropriate effectors according to your protocol; do not add treatment to the positive and negative control cells.

7.1.4 **Negative control** (cells not exposed to the Protein Label or treatment), **positive control** (cells incubated with 1X Protein Label only).

7.1.5 To use Cycloheximide as an inhibitor of protein synthesis, dilute it 1:100 in the culture medium and incubate the cells for 30 minutes at a 37°C. Remove the drug-containing media.

7.1.6 For immobilized suspension cells: Centrifuge the plate at 300 x g (or the lowest centrifuge setting) for 5 minutes to deposit the cells onto the surface.

7.1.7 Tilt the plate and gently remove the media with a pipette tip. It is important to avoid excessive centrifugation speeds, which can damage the cells.

Δ Note: Make note of the place that is used, and perform subsequent aspirations from the same place. Pellet the suspension cells at 300 x g for 5 min throughout the entire protocol!

7.1.8 Replace the media with fresh aliquots containing Protein Label (400X) diluted to 1X final concentration and tested compound or Cycloheximide.

7.1.9 Add Protein Label to the positive control cells. Incubate the cells for additional 0.5 - 2 hours in a 37°C incubator, or for the period of time required by your experimental protocol.

Δ Note: For drug and Protein Label **co-incubation**, dilute Protein Label directly into the drug or Cycloheximide treated cells, do not change the media.

7.1.10 Terminate the experiment by removal of the culture medium and rinse the cells once with 100 µL of PBS, discard the supernatant. Proceed to the Fixation and Permeabilization.

7.2 Fixation and permeabilization:

7.2.1 **For adherent cells:** Add 100 µL of Fixative Solution I to each well and incubate the cells for 15 minutes at room temperature protected from light. Remove the Fixative Solution I and wash the cells once with 200 µL of 1X Wash Buffer IV. Remove the Wash Buffer IV and add 100 µL of 1X Permeabilization Buffer. Incubate the cells for 10 minutes at room temperature and remove the Permeabilization Buffer.

7.2.2 **For suspension cells**: Re-suspend the cells in 100 µL of Fixative Solution I and incubate for 15 min at RT protected from light. Centrifuge cells at 900 x g for 5 minutes and remove the Fixative Solution I. Wash the cells once with 200 µL of 1X Wash Buffer IV. Centrifuge cells at 900 x g for 5 minutes and discard the supernatant. Re-suspend the cells in 100 µL of 1X Permeabilization Buffer. Incubate the cells for 10 min at RT and remove the Permeabilization Buffer.

7.3 Protein Reaction and total DNA staining:

7.3.1 **Reaction cocktail:** Prepare reaction cocktail according to the table below. Volumes should be multiplied by number of samples and reagents added in the exact order. Use the reaction cocktail within 15 minutes of preparation.

Δ Note: Cells should be protected from light during, and following the reaction and DNA staining**.**

	Amount per reaction
PBS	93 µL
100X Copper Reagent	1 µL
Fluorescent Azide II	1 µL
Reducing Agent Solution	5 µL

7.3.2 **Protein Reaction:** Add 100 µL of 1X Reaction cocktail to each sample and incubate the cells for 30 min at RT protected from light.

7.3.3 Centrifuge cells at 900 x g for 5 minutes and remove the reaction cocktail. Wash cells twice in 200 µL of Wash Buffer IV. Centrifuge cells at 900 x g for 5 minutes and remove the Wash Buffer IV after each wash. Re-suspend the cells in 100 µL of PBS.

7.3.4 Proceed to DNA staining. If no DNA staining is desired, proceed to Microscopic or FACS analysis.

7.3.5 **DNA staining:** Prepare 1X dilution of DAPI and add 100 µL per well. Incubate the cells for 20 minutes at room temperature, or refrigerate at 4 °C protected from light.

7.3.6 Centrifuge cells at 900 x g for 5 minutes and remove the DNA stain solution; wash the cells once with 100 µL of PBS.

Δ Note: Cells are compatible with all methods of slide preparation including wet mount or prepared mounting media.

7.4 Fluorescence Microscope imaging:

7.4.1 Analyze samples for green fluorescence generated by labeled Protein and for blue fluorescence by nuclear DNA.

7.4.2 **FACS analysis:** Harvest the cells by preferred method and wash with 0.5 mL of ice-cold PBS. Centrifuge cells at 900 x g for 5 minutes and re-suspend the pellets with 100 µL of ice-cold PBS. Analyze samples for green fluorescence generated by de novo synthesized protein during click reaction.

8. Typical Data

Typical data provided **for demonstration purposes only**.

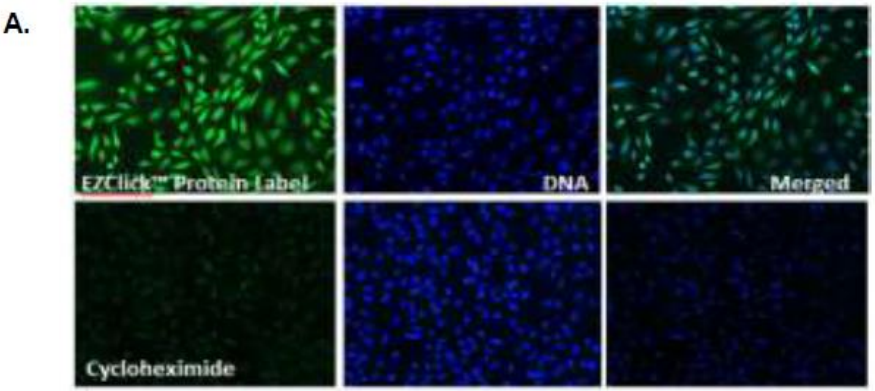


Figure 1. **Inhibitory effect of Cycloheximide on nascent polypeptides synthesis**. HeLa (10^5 cells/ mL) and Jurkat ($1X10^6$ cells/mL) cells respectively were pre-treated with vehicle or Cycloheximide for 30 min at 37°C. Subsequently, cells were incubated for additional 30 min with fresh aliquots of media containing either Protein Label or Protein Label and Cycloheximide. Cells were then processed and analyzed by Microscopy and FACS according to the kit protocol. **(A)** Green fluorescence (upper panel) corresponds to *de novo* synthesized polypeptides whereas bottom panel shows the inhibitory effect of Cycloheximide on protein biosynthesis. Nuclear staining in both panels confirms that green signal is a result of Protein Label incorporation.

B.

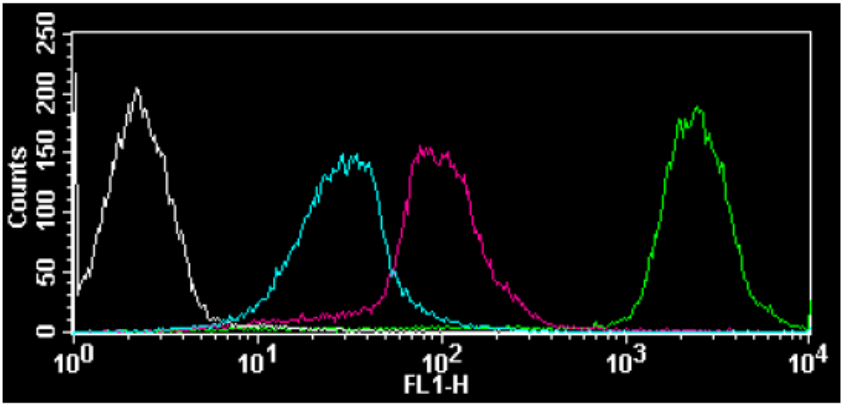


Figure 2. Inhibitory effect of Cycloheximide on nascent polypeptides synthesis. HeLa (10^5 cells/ mL) and Jurkat ($1X10^6$ cells/mL) cells respectively were pre-treated with vehicle or Cycloheximide for 30 min at 37°C. Subsequently, cells were incubated for additional 30 min with fresh aliquots of media containing either Protein Label or Protein Label and Cycloheximide. Cells were then processed and analyzed by Microscopy and FACS according to the kit protocol. **(B)** FACS analysis of negative control (white), background (Azide only, blue), positive control (Protein Label, green) and Cycloheximide-treated (pink) cell populations. Signal measured in FL-1 channel clearly shows the inhibitory effect of Cycloheximide on nascent polypeptides synthesis.

9. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)