Version 4a, Last updated 8 December 2025

ab235634
Protein Synthesis Assay Kit (Red)

For the measurement of nascent protein biosynthesis 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

Protein Synthesis Assay Kit (Red) (ab235634) 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. The kit provides sufficient materials for 100 assays to detect nascent proteins synthesized under various physiological conditions, and Cycloheximide, an inhibitor of protein synthesis that serves as an experimental control.

Seed cell suspension directly into tissue culture vessels.

Dilute the Cycloheximide in culture media and incubate for 30 min at 37°C.

Replace media with fresh media containing Protein Label and test compound or Cycloheximide. Add Protein Label to the positive control cells. Incubate for 0.5-2 hours at 37°C.

Remove culture medium and rinse cells with PBS.

Add Fixative Solution and incubate for 15 min at RT protected from light. Wash cells and incubate with Permeabilization Buffer for 10 min at RT.

Incubate cells with 1X Reaction cocktail for 30 minutes at RT protected from light. Wash and proceed to DNA staining if desired.

Analyze samples for by Fluorescence Microscope or FACS.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. 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.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
10X Wash Buffer IV	25 mL	-20°C	4°C
Fixative Solution I	10 mL	-20°C	-20°C
10X Permeabilization Buffer	25 mL	-20°C	4°C
400X Protein Label	25 µL	-20°C	-20°C
100X Copper Reagent	100 µL	-20°C	-20°C
100X Fluorescent Azide I	100 µL	-20°C	-20°C
Reducing Agent	1 vial	-20°C	-20°C
1000X Total DNA Stain	10 µL	-20°C	-20°C
100X Cycloheximide	10 µL	-20°C	-20°C

3. 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).
- Fluorescence microscope capable of excitation and emission at 440/490 and 540/580 nm respectively.

4. 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.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 10X Wash Buffer

1. Thaw at 37°C to dissolve completely.
2. Dilute the 10X stock 1:10 in sterile water. Mix well.

5.2 Fixative Solution I

1. Ready to use as supplied.

5.3 10X Permeabilization Buffer

1. Thaw at 37°C to dissolve completely.
2. Dilute the 10X stock 1:10 in sterile water. Mix well.

5.4 400X Protein Label

1. Ready to use as supplied.
2. While in use, keep on ice and minimize light exposure.

5.5 100X Copper Reagent

1. Ready to use as supplied.
2. While in use, keep on ice and minimize light exposure.

5.6 100X Fluorescent Azide I

1. Ready to use as supplied.
2. While in use, keep on ice and minimize light exposure.

5.7 20X Reducing Agent

1. Resuspend the reducing agent with 560 µL Type I water. Mix well and store solution at -20°C protected from light.

   Δ Note: For older lots (20X Reducing Agent 500 µL), ready to use as provided. Store solution at -20°C protected from light.
2. While in use, keep on ice and minimize light exposure.

5.8 1000X Total DNA Stain

1. Ready to use as supplied.
2. While in use, keep on ice and minimize light exposure.

5.9 100X Cycloheximide

1. Ready to use as supplied.
2. While in use, keep on ice and minimize light exposure.

6. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

Δ **Note:** This assay was developed with HeLa (adherent) and Jurkat (suspension) cells and can be modified for any cell line.

Δ **Note:** 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. 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.

6.1 Labeling of control and experimental cells

1. Obtain cell suspension of desired density and seed directly into tissue culture vessels, or on coverslips for high resolution microscopy.
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. Gently remove the gelatin solution and seed your cells. Allow the cells to recover overnight before the treatment.
3. Next day, treat the cells with appropriate effectors according to your protocol.

Δ **Note:** Do not add treatment to the positive and negative control cells.

- Negative control: cells not exposed to the Protein Label or treatment.
- Positive control: Cells incubated with 1X Protein Label only.

4. Replace the media with fresh aliquots containing Protein Label (400X) diluted to 1X final concentration alone, with tested compound, or with Cycloheximide. To use the Cycloheximide as an inhibitor of protein synthesis, dilute it 1:100 in the culture medium. Add Protein Label alone to the positive control cells. Add Protein Label with Cyclohexamide or test compounds to the experimental cells. Incubate the cells for additional 0.5-2 hours in a 37°C incubator, or for the period of time as required by your experimental protocol.
5. Terminate the experiment by removing the culture medium and wash the cells once with 100 µl of PBS and discard the supernatant. For immobilized suspension cells: Centrifuge the plate at 300 x g (or the lowest centrifuge setting) for 5 min to deposit the cells onto the surface. 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. Make note of the place that is used for aspiration, and perform subsequent aspirations from the same place.

6.2 Fixation and Permeabilization

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 and wash the cells once with 200 µL of 1X Wash Buffer IV/Wash Buffer, then aspirate the wash. Add 100 µL of 1X Permeabilization Buffer and incubate the cells for 10 minutes at room temperature. Remove the Permeabilization Buffer.
2. For suspension cells: Re-suspend the cells in 100 µL of Fixative Solution I and incubate for 15 minutes at room temperature protected from light. Centrifuge cells at 900 x g for 5 minutes and aspirate 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, discard the supernatant, and re-suspend the cells in 100 µL of 1X Permeabilization Buffer. Incubate the cells for 10 minutes at room temperature. Centrifuge cells at 900 x g for 5 minutes and remove the Permeabilization Buffer.

6.3 Protein reaction and total DNA staining

1. Prepare 1X 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.

Component	Reaction Mix (µL)
PBS	93 µL
100 Copper Reagent	1 µL
100X Fluorescent Azide I	1 µL
Reducing Agent Solution	5 µL

2. Add 100 µL of 1X Reaction cocktail to each sample and incubate the cells for 30 minutes at room temperature protected from light. Centrifuge cells at 900 x g for 5 minute and aspirate the reaction cocktail. Wash cells in 200 µL of Wash Buffer IV/Wash Buffer. Centrifuge cells at 900 x g for 5 minutes. Aspirate the Wash Buffer IV/Wash Buffer and suspend the cells in 100 µL of PBS.

Δ Note: If no DNA staining is desired, proceed to Microscopic or FACS analysis.

3. DNA staining: Prepare 1X dilution of Total DNA Stain and add 100 µL per well. Incubate the cells for 20 minutes at room temperature, or refrigerate at 4 °C protected from light. Centrifuge cells at 900 x g for 5 minute and remove the DNA stain solution. Wash the cells once with 200 µL of PBS.

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

6.4 Fluorescence Microscope and FACS analysis

1. Fluorescence Microscope analysis: Analyze samples for red fluorescence generated by labeled Protein and for green fluorescence by nuclear DNA.
2. FACS analysis: Harvest the cells by preferred method and wash with 0.5 mL of ice-cold PBS. Re-suspend the pellets in 100 µL of ice-cold PBS and analyze samples for red fluorescence generated by *de* novo synthesized protein during click reaction.

7. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

8. Typical Data

Data provided **for demonstration purposes only**.

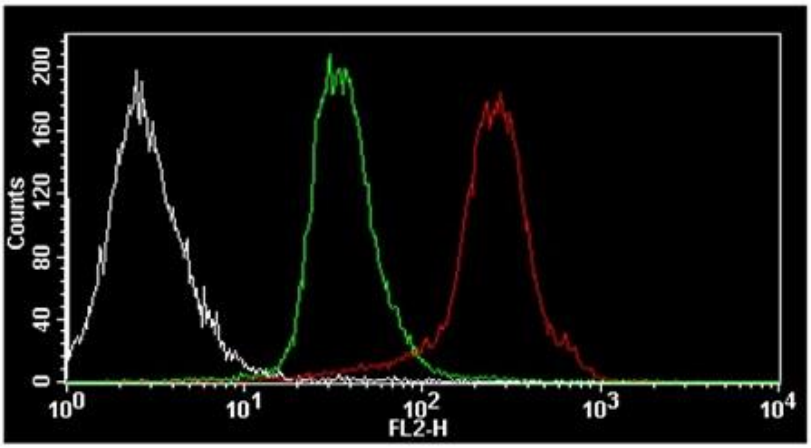

Figure 1. Analysis of protein biosynthesis in presence of Cycloheximide. Jurkat cells (1X10^6 cells/mL) were pre-incubated either with vehicle or 1X Cycloheximide for 30 minutes at 37°C incubator to suppress protein synthesis followed media exchange. Cells were then incubated either with culture medium alone (white) or 1X Protein Label (red) or 1X Cycloheximide (green) for 30 minutes in the cell culture incubator. The cells were processed for protein synthesis detection by FACS according to the included protocol. Fluorescence measured in FL-2 channel clearly shows the inhibitory effect of Cycloheximide on nascent poppypeptides synthesis.

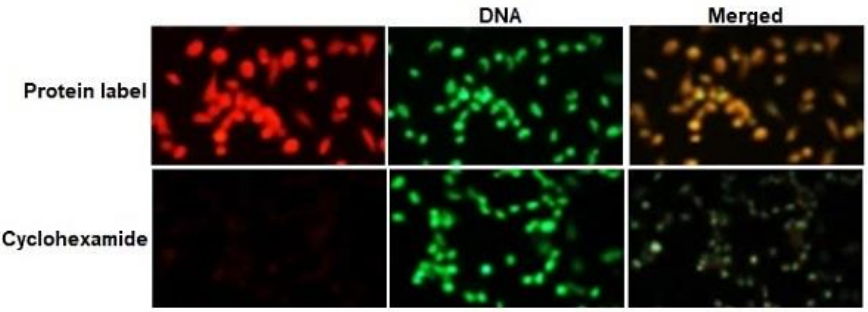

Figure 2. Red fluorescence (upper panel) corresponds to *de novo* synthesized polypeptides whereas bottom panel shows the inhibitory effect of Cycloheximide on protein biosynthesis. Nuclear staining in both panels confirms that red signal is a result of Protein Label incorporation.

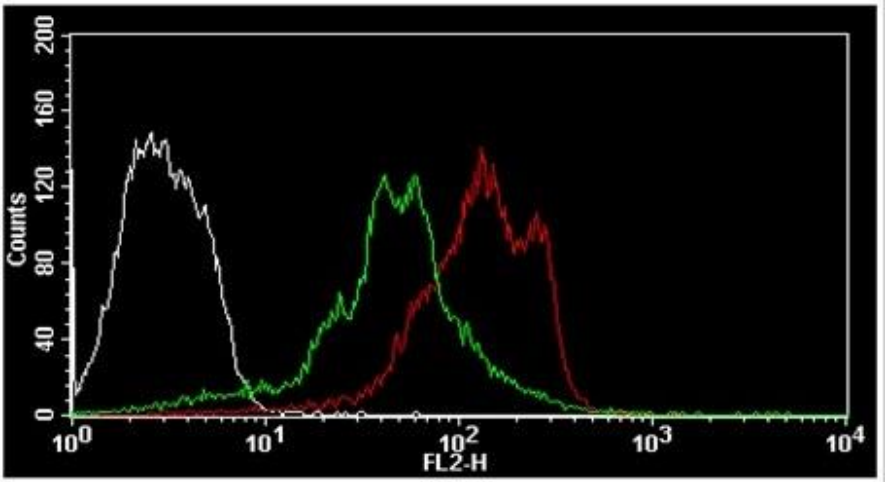

Figure 3. FACS analysis of negative control (white), positive control (Protein Label, red) and Cycloheximide-treated (green) cell populations. Signal measured in FL-2 channel clearly shows the inhibitory effect of Cycloheximide on nascent polypeptides synthesis.

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)