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ab273286
Protein Synthesis Assay Kit
(Green, Microplate)

View Kit datasheet: https://www.abcam.com/ab273286
(use https://www.abcam.cn/ab273286 for china, or
https://www.abcam.co.jp/ab273286 for Japan)

For the determination of protein synthesis in adherent/suspension cells.

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

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1. Overview

Protein Synthesis Assay Kit (Green, Microplate) (ab273286) utilizes a novel chemical method based on alkyne analog of puromycin, O-Propargyl-puromycin (OP-puro). OP-puro stops translation by forming covalent conjugates with the 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 (Ex/Em = 494/521 nm). OP-puro does not require methionine-free conditions and can be used to label nascent proteins directly in the cell culture medium.

This kit provides sufficient materials for 100 simple and specific assays to detect nascent proteins synthesized under various physiological conditions in real-time, and in the presence of Cycloheximide, an inhibitor of protein synthesis that serves as a control.

2. Protocol Summary

Culture cells of choice

Label the control and experimental cells. Prepare Negative Control (no Protein Label, no Reaction Cocktail), Positive Control (cells incubated with 1X Protein Label only) and Background Control (Reaction Control only) cells

Fix and permeabilize cells

Add Reaction Cocktail to appropriate cells

Staining

Remove excess stain and resuspend cells in PBS

Analyze cells immediately in the plate reader at Ex/Em 494/521 nm in end point mode

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
10X Wash Buffer IV	25 mL	-20°C
Fixative Solution I	10 mL	-20°C
10X Permeabilization Buffer	25 mL	-20°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

7. 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)
- 6-, 12-, 24-, or 96-well clear plates should be used only for cell culture. The measurement of fluorescence should be performed in white opaque plates. Alternatively, sterile opaque plates can be used for both, culturing and measurements
- Multi-well spectrophotometer and Fluorescence microscope (optional) capable of measuring Excitation and Emission = 494/521 nm spectra

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.
- Hot plate/dry heat block or microplate incubator

9. Reagent Preparation

Briefly centrifuge small vials prior to opening.

9.1 10X Wash Buffer IV:
Dilute the 10X stocks 1:10 in sterile water, mix well. Store at 4°C.

9.2 10X Permeabilization Buffer:
Dilute the 10X stocks 1:10 in sterile water, mix well. Store at 4°C.

9.3 Fixative Solution I:
Ready to use, after opening store at 4°C, protected from light.

9.4 Reducing Agent:
Resuspend 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.

All other components should be stored at -20°C protected from light. While in use, keep on ice and minimize light exposure.

10. Assay Protocol

- This assay was developed with Jurkat (suspension) and HeLa (adherent) cells and can be modified for any cell line.
- The following protocol has been optimized for a 96-well white opaque plate at 1 X 10^6 cells per well, using fewer cells per well or clear plate will result in decreased signal.
- We suggest testing growth conditions, cell number per well and several concentrations of the Protein Label, to find the best experimental design for your cell type.
- The assay volume is 100 µl; adjust volumes accordingly for other plate formats.
- Avoid stressing cells by washes or temperature changes prior to incubation with Protein Label.
- All steps should be carried out at room temperature (RT) unless otherwise specified.
- Equilibrate all buffers to RT prior to the experiment.

Labeling of control and experimental cells

10.1 Plate suspension or adherent cells at a desired density and allow for overnight recovery before treatment. Ensure that adherent cells are sub-confluent.

10.2 Include appropriate controls and account for cell loss during the processing: **Negative Control** (cells not exposed to the Protein Label or treatment), **Background Control** (cells treated with Reaction Cocktail only), **Positive Control** (cells incubated with 1X Protein Label only).

10.3 To use Cycloheximide as an inhibitor of protein synthesis, dilute it 1:100 in the culture medium and incubate cells for 30 mins at 37°C, remove the media. For Suspension Cells: Centrifuge the plate at 300 *x g* (or the lowest centrifuge setting) for 5 mins at RT to pellet the cells. Tilt the plate and gently remove the media with a pipette tip. Avoid excessive centrifugation speeds, which can damage the cells. Use these centrifugation settings throughout the entire protocol.

10.4 Replace the media with fresh aliquots containing 1X Protein Label and tested compound(s), condition(s) or Cycloheximide.

10.5 Add Protein Label to the Positive Control cells. Incubate the cells for additional 0.5-24 hours, or 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.

10.6 Terminate the experiment by removal of the culture media. Harvest the suspension cells by centrifugation. Optional: detach adherent cells (e.g. trypsinize and quench with media), and harvest by centrifugation.

10.7 Wash the cells once with 100 µl of PBS, discard the supernatant and proceed to the Fixation and Permeabilization.

Fixation and Permeabilization

For adherent and suspension cells:

10.8 Add 100 µl of Fixative Solution I to each well and incubate the cells for 15 mins at RT protected from light.

10.9 Remove the Fixative Solution I and wash cells once with 100 µl of 1X Wash Buffer IV, remove wash solution.

10.10 Add 100 µl of 1X Permeabilization Buffer and incubate the cells for 10 mins at RT.

10.11 Remove the Permeabilization Buffer and replace it with a 20 µl of fresh aliquot of Permeabilization Buffer.

10.12 Proceed to Protein reaction.

Protein reaction and total DNA staining:

- Volumes should be multiplied by number of Samples and reagents added in the exact order.
- Use the Fluorescent Azide II Reaction Cocktail within 15 mins of preparation.
- Cells should be protected from light during and following the reaction and DNA staining.

10.13 Prepare a 1X Reaction Cocktail according to the table below:

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

10.14 Add 100 µl of 1X Reaction Cocktail to each sample and incubate the cells for 30 mins at RT protected from light.

10.15 Remove the reaction cocktail and wash cells three times in 100 µl of Wash Buffer IV.

10.16 Suspend the cells in 100 µl of Wash Buffer IV.

10.17 For detection cells must be analyzed **immediately** in the plate reader at Ex/Em 494/521 nm in end point mode to determine change in fluorescence of compounds and controls after

background subtraction or imaged with fluorescence microscope directly in the plate following DNA staining.

10.18 For the DNA staining prepare 2X dilution of DAPI and add 100 µl per well. Incubate the cells for 20 mins at RT or refrigerate at 4 °C protected from light.

10.19 Remove the 1000X DAPI/DNA stain and re-suspend the cells in 100 µl of PBS prior to imaging.

10.20 Analyze samples for green fluorescence generated by de novo synthesized protein and for blue fluorescence by nuclear DNA.

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

10.21 When preparing an Azide Fluorescence Curve to increase the accuracy of your data, an azide fluorescence curve should be prepared from the same cell suspension in parallel to the experimental treatment for each cell line and condition.

10.22 In a 96-well white opaque plate, prepare a series of dilutions of your cell suspension starting with the same volume and number of cells as in the experimental wells. Dilute the cells by factor of 2. Optional: To minimize the error, aliquot at least 3 wells per dilution.

10.23 Measure fluorescence and calculate average for each dilution, subtract the background value.

10.24 Plot the Azide Fluorescence Curve to obtain fluorescence per cell number and the detection limit for your assay.

10.25 Also, standard curve of Fluorescent Azide II concentration per well can be prepared in the same manner to obtain the least detectable amount of azide for your experiment.

11. Typical Data

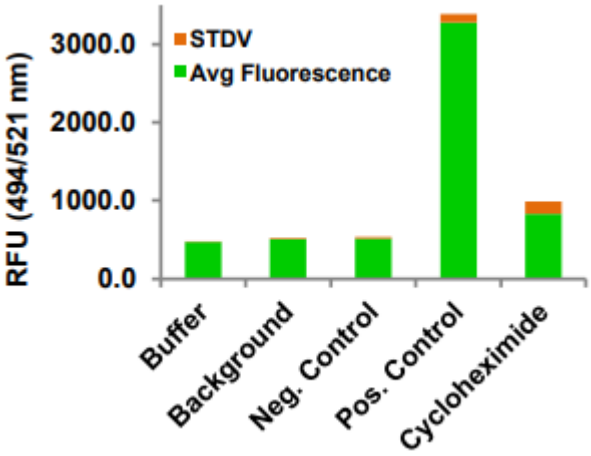

Figure 1: Plate reader analyses on Jurkat cells (1X10^6 cells/well) with controls and Cycloheximide treatment. Average fluorescence +/- standard deviation plotted for 3 replicates per condition.

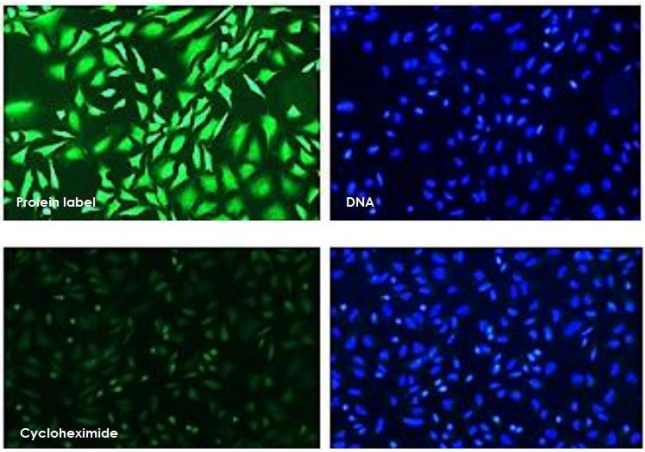

Figure 2: Metabolic labeling of protein on proliferating HeLa cells. 1 x 10^5 cells incubated overnight with fresh aliquots of media containing Protein Label: Upper panel corresponds green fluorescence of de novo synthesized peptides. The lower panel shows cells treated with 1X Cycloheximide. Nuclear staining in both panels confirms that green signal is a result of Protein Label incorporation.

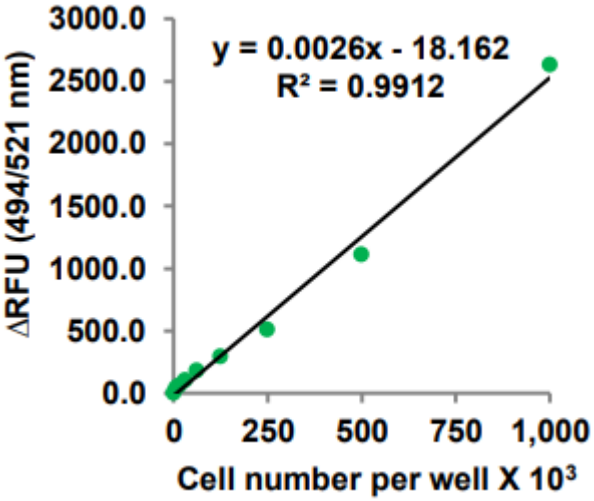

Figure 3. Fluorescence azide curve of Jurkat cells prepared for this assay. Detection limit corresponds to about 31,250 of Jurkat cells per well. A new curve must be obtained for each experiment and the cell line.

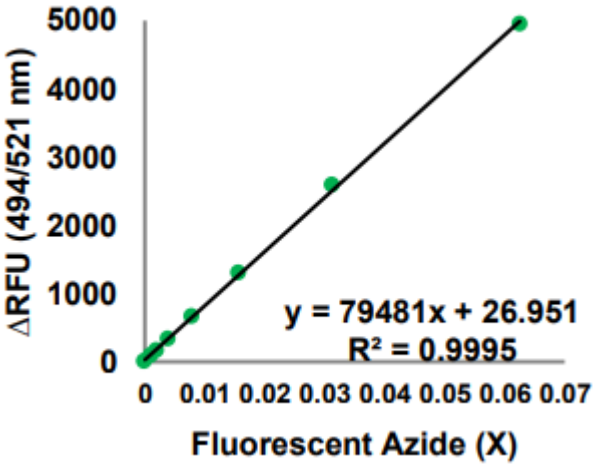

Figure 4. Azide Fluorescence Curve in 0-0.1 X range. This is reference data and it should not be used to interpret actual results. Your data will depend on the cell type and tested compound.

12. FAQ / Troubleshooting

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

13. 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)