Protein Synthesis Assay Kit

Item No. 601100

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Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	100 Tests Quantity/Size	Storage
10009866	Cell-Based Assay Wash Buffer	2 vials/100 ml	RT
600744	Cell-Based Assay TBS (10X)	1 vial/10 ml	RT
601101	O-Propargyl-Puromycin Stock Solution	1 vial/25 µl	-20°C
601102	Copper Sulfate Solution	1 vial/100 µl	-20°C
10009899	Cell-Based Assay Fixative	1 vial/10 ml	RT
601103	Ascorbic Acid Solution	1 vial/2 ml	-20°C
601104	Cell-Based Assay 5 FAM-Azide Stock Solution	1 vial/25 µl	-20°C
601105	Cell-Based Assay Cycloheximide	1 vial/50 µl	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A 6-, 12-, 24-, or 96-well plate for culturing cells.
2. A flow cytometer, fluorescence microscope, or plate reader equipped with laser/fluorescent filters capable of detecting 5-carboxyfluorescein (5 FAM) at the excitation and emission wavelengths of 485 and 535 nm, respectively.
3. A plate centrifuge.

INTRODUCTION

Background

Measurement of protein synthesis has previously been accomplished by using radioactive tracers, such as tritiated phenylalanine or [^{35}S]-methionine. Radioactive approaches can be cumbersome and expensive. O-Propargyl-puromycin (OPP) is a puromycin analog containing an alkyne moiety. It is cell-permeable and, once inside cells, it incorporates into the C-terminus of translating polypeptide chains, thereby stopping translation. The truncated C-terminal alkyne-labeled proteins can subsequently be detected *via* copper-catalyzed click chemistry.[1]

About This Assay

Cayman's Protein Synthesis Assay Kit includes OPP as a probe for labeling translating polypeptide chains and 5 FAM-Azide for subsequent detection of OPP-labeled proteins. The reagents provided in the kit are sufficient to run 20 samples when using flow cytometry or 100 samples when using a 96-well plate format.

PRE-ASSAY PREPARATION

NOTE: 5 FAM-Azide is light sensitive. Do not expose to direct intense light.

Reagent Preparation

1. **Assay Buffer (1X) Preparation**

   Dilute the Cell-Based Assay TBS (10X) (Item No. 600744) with 90 mls of distilled water. Mix well. The diluted Assay Buffer should be stable for at least one year when stored at room temperature.

2. **O-Propargyl-Puromycin (OPP) Working Solution Preparation**

   Prepare an OPP Working Solution by adding 2.5 µl of OPP Stock Solution (Item No. 601101) to 1 ml of the culture medium used for your cells. Mix well. Prepare this solution immediately before adding to the samples. The OPP Stock Solution (Item No. 601101) is sufficient to make 10 ml of OPP Working Solution.

3. **Cell-Based Assay 5 FAM-Azide Staining Solution Preparation**

   To make 10 ml of 5 FAM-Azide Staining Solution, sufficient for use in one 96-well plate, add the following to 10 ml of Assay Buffer (1X). Prepare the solution immediately before use.

   10 µl of Cell-Based Assay 5 FAM-Azide Stock Solution (Item No. 601104)

   10 µl of Copper Sulfate Solution (Item No. 601102)

   100 µl of Ascorbic Acid Solution (Item No. 601103)

NOTES

- 5 FAM-Azide is light sensitive. All staining procedures must be performed without direct exposure to intense light. Therefore, incubations need to be done in the dark.
- For all assay protocols, on pages 7-10, it is imperative that samples be analyzed immediately following completion of the staining.

ASSAY PROTOCOL

NOTE: The following protocol has been optimized for 1 x 10^6 cells/sample. Optimal conditions may depend on the cell type.

Flow Cytometry

1. Culture and treat cells according to your experimental protocol. To use the Cell-Based Assay Cycloheximide (Item No. 601105) included in the kit as a negative control, dilute 1:1,000 in the culture medium and incubate the cells in the cycloheximide-containing medium for 30 minutes at 37°C.
2. Collect the cells in a test tube and spin at 400 x g for five minutes. Carefully aspirate the supernatant.
3. Resuspend the cells in 0.5 ml of OPP Working Solution or in 0.5 ml of OPP Working Solution containing test compounds or the negative control at the concentrations used in Step 1. Mix well to ensure separation of individual cells. Incubate the cells for 30 minutes to two hours at 37°C. A 30 minute incubation should be sufficient for OPP labeling of translating peptides.
4. Spin down the cells at 400 x g for five minutes and carefully aspirate the supernatant.
5. Resuspend the cells in 0.5 ml of Cell-Based Assay Fixative (Item No. 10009899). Mix well to ensure separation of individual cells. Incubate the cells at room temperature for five minutes.
6. Spin down the cells at 400 x g for five minutes and carefully aspirate the supernatant.
7. Resuspend the cells in 1 ml of the Cell-Based Assay Wash Buffer (Item No. 10009866). Mix well to ensure separation of individual cells. Incubate the cells at room temperature for five minutes.
8. Spin down the cells at 400 x g for five minutes and carefully aspirate the supernatant.
9. Repeat steps 7 to 8 two more times.

10. Resuspend the cells in 1 ml of 5 FAM-Azide Staining Solution and incubate the cells in the dark at room temperature for 30 minutes.
11. Spin down the cells at 400 x g for five minutes and carefully aspirate the supernatant.
12. Resuspend the cells in 1 ml of the Cell-Based Assay Wash Buffer. Mix well to ensure separation of individual cells. Incubate the cells at room temperature for five minutes.
13. Spin down the cells at 400 x g for five minutes and carefully aspirate the supernatant.
14. Repeat Steps 12 to 13 two more times.
15. *Optional: Stain for surface markers according to your typical antibody staining protocols. Please note that the fixation of cells can negatively impact some surface markers, so we recommend testing your antibodies on cells fixed according to this protocol.*
16. Resuspend the cells in 1 ml Assay Buffer (1X) and analyze the cells with a flow cytometer capable of detecting FITC at excitation/emission = 483 nm/525 nm, usually in the FL1 channel of a flow cytometer. The cells must be analyzed immediately.

Fluorescence Microscopy or Plate Reader Detection

The following protocol is designed for a 96-well clear bottom, black, cell culture plate. We recommend that the cell density be 1 x 10^4-5 x 10^4 cells/ml. Optimal conditions will depend on the cell type.

1. Culture and treat cells according to your experimental protocol. To use the Cell-Based Assay Cycloheximide (Item No. 601105) included in the kit as a negative control, dilute 1:1,000 in the culture medium and incubate the cells in the cycloheximide-containing medium for 30 minutes at 37°C.
2. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate the supernatant.
3. Add 100 µl of OPP Working Solution or 100 µl of OPP Working Solution containing test compounds or the negative control at the concentrations used in Step 1 to the corresponding wells. Incubate the cells for 30 minutes to two hours at 37°C.
4. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate the supernatant.
5. Add 100 µl of Cell-Based Assay Fixative (Item No. 10009899) to each well. Incubate the cells at room temperature for five minutes.
6. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate the supernatant.
7. Add 100 µl of Cell-Based Assay Wash Buffer (Item No. 10009866) to each well. Incubate the cells at room temperature for five minutes.
8. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate the supernatant.
9. Repeat Steps 7-8 two more times.
10. Add 100 µl of 5 FAM-Azide Staining Solution to each well and incubate the cells in the dark at room temperature for 30 minutes.
11. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate the supernatant.

12. Add 100 µl of Cell-Based Assay Wash Buffer to each well. Incubate the cells at room temperature for five minutes.
13. Centrifuge the plate for five minutes at 400 x g at room temperature. Carefully aspirate the supernatant.
14. Repeat Steps 12-13 two more times.
15. Add 100 µl of Assay Buffer (1X) to each well and examine the cells by fluorescence microscopy or a fluorescent plate reader using a filter designed to detect FITC (excitation/emission = 485/535 nm). Cells must be analyzed immediately.

PERFORMANCE CHARACTERISTICS

Representative Fluorescence Detection Results

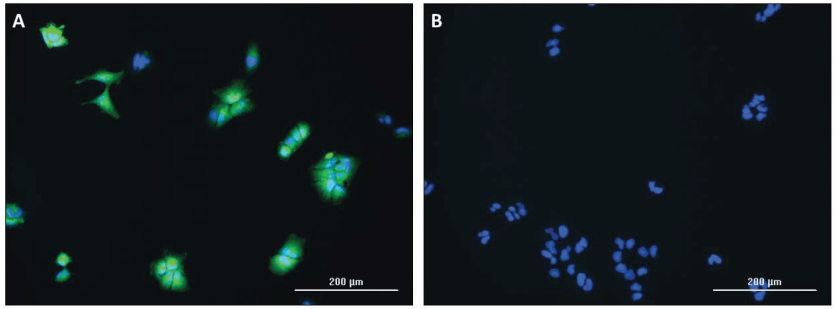

Figure 1. Cycloheximide inhibits mRNA translation to polypeptides. Huh7 cells were plated at a density of 2 x 10^3 cells/well in a 96 well plate and cultured overnight. The next day, cells were treated with vehicle (*Panel A*) or 50 ug/ml cycloheximide (*Panel B*) for 30 minutes before staining according to the protocol on pages 9 and 10 of this kit booklet. After step 12, nuclei were stained with DAPI (Item No. 601361) at a dilution of 1:600 to aid in visualization before continuing with remaining wash steps. Cells were imaged using BioTek's Cytation™ 5 Cell Imaging Multi-Mode Reader. The 10X objective and two LED filter cubes were used: Violet (blue nuclear stain) and FITC (green translating polypeptide chains).

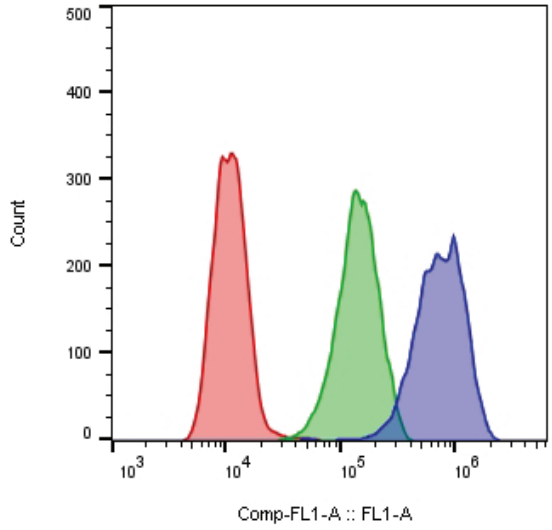

Figure 2. Cycloheximide inhibits mRNA translation to polypeptides, as measured by flow cytometry. THP-1 cells were plated at a density of 1 x 10^6 cells/well in a 6-well plate. The next day, cells were treated with vehicle or 50 µg/ml cycloheximide for 30 minutes in a CO_2 incubator at 37°C. Cells were then incubated in either culture medium alone (Red) or OPP without cycloheximide (Blue) or OPP with 50 µg/ml cycloheximide (Green) for 30 minutes in a cell culture incubator. The cells were then processed for detection of protein synthesis according to the protocol described in the **Flow Cytometry** section.

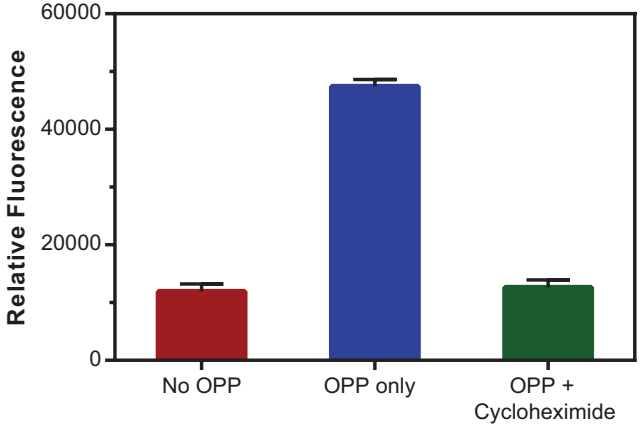

Figure 3. Cycloheximide inhibits mRNA translation to polypeptides, as measured by a plate reader. HeLa cells were plated at a density of 5 x 10^4 cells/well in a 96-well clear bottom black plate. The next day, cells were left untreated or treated with 50 µg/ml cycloheximide for 30 minutes in a CO_2 incubator at 37°C. Cells were then incubated in either culture medium alone, culture medium containing OPP or culture medium containing OPP and 50 µg/ml cycloheximide for 30 minutes in a cell culture incubator. The cells were then processed for detection of protein synthesis according to the protocol described in the **Fluorescence Microscopy and Plate Reader Detection** section.

RESOURCES

Reagent	Procedure
Assay Buffer (1X)	Dilute Cell-Based Assay TBS (10X) 1:10 with distilled water
O-Propargyl-Puromycin (OPP) Working Solution	Add 2.5 µl of OPP Stock Solution to 1 ml of culture medium.
Cell-Based Assay 5 FAM-Azide Staining Solution	Add the following to 10 ml of Assay Buffer (1X): • 10 µl of Cell-Based Assay 5 FAM-Azide Stock Solution • 10 µl of Copper Sulfate Solution • 100 µl of Ascorbic Acid Solution

Table 1. Assay reagent preparation summary

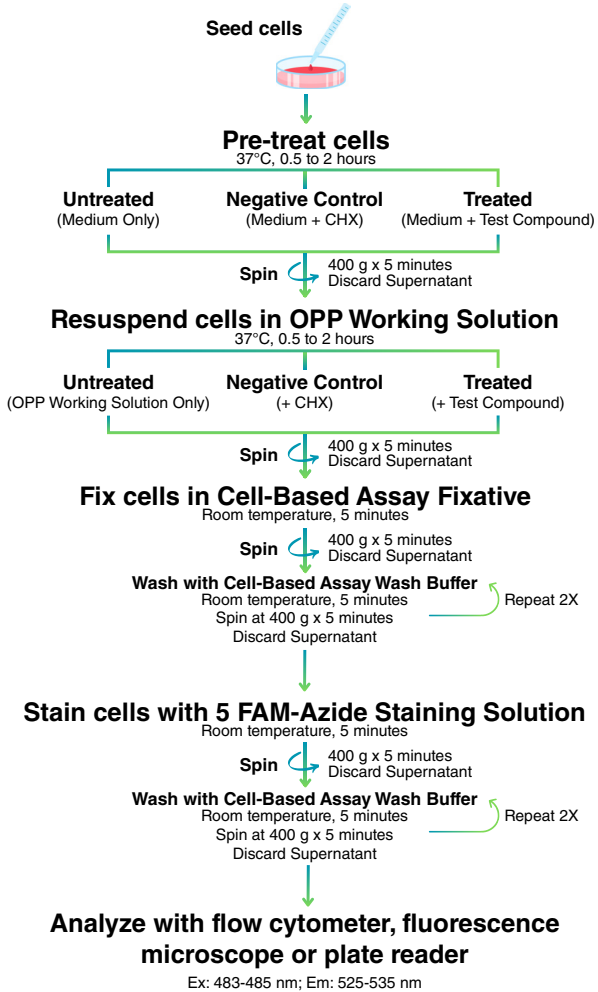

Figure 4. Assay summary

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal detected for all treatments including vehicle control	Cells are not healthy	Use only healthy cells
High level of fluorescence intensity in all samples, including no OPP controls	Insufficient wash to remove FAM-Azide Staining Solution	Be sure to complete all the wash steps as described in the protocols

Reference

1. Liu, J., Xu, Y., Stoleru, D., *et al.* Imaging protein synthesis in cells and tissues with an alkyne analog of puromycin. *Proc. Natl. Acad. Sci. USA* **109(2)**, 413-418 (2012).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.