7-AAD Cell Viability Assay Kit

Item No. 10009856

Customer Service 800.364.9897 * **Technical Support** 888.526.5351
www.caymanchem.com

TABLE OF CONTENTS

Materials Supplied

Item Number	Item	100 Tests Quantity/Size	Storage
400201	Cell-Based Assay 7-AAD Staining Stock Solution (1,000X)	1 vial/50 µL	4°C
400202	7-AAD Assay Fixative/Actinomycin D Solution	1 vial/50 mL	4°C
10009322	Cell-Based Assay Buffer Tablet	4 Tablets	Room Temperature

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 laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

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

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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-, or 24-well plate for culturing cells.
2. A flow cytometer equipped with a 488 nm excitation laser.

INTRODUCTION

Background

Cell viability is an important parameter to measure when determining cell responses to endogenous factors, such as hormones and growth factors, or exogenous factors such as cytotoxic drugs and environmental stress. Evaluation of cell viability is essential in cell biology and drug discovery.[1] There are a number of assays available that detect cytotoxicity or cell viability, independent of mechanism. Most of these assays assess cell viability by measuring plasma membrane permeability.[2] Live cells with intact membranes are distinguished by their ability to exclude fluorescent dyes such as propidium iodide and 7-amino actinomycin D (7-AAD) that penetrate dead or damaged cells to label DNA. Like propidium iodide, 7-AAD is a fluorescent DNA intercalator which binds to double stranded DNA. It can be excited by an argon-ion laser (excitation at 488 nm, emission at 650 nm).[2] Dead cells labeled by these dyes can be measured quantitatively using a flow cytometer. Thus, the cytotoxicity of chemical compounds, drugs, or cellular interactions can be assessed. Although 7-AAD fluorescence is less intense than that of propidium iodide, its emission is at a higher wavelength and thus has minimal spectral overlap with PE or FITC. This makes 7-AAD preferable as a viability marker when FITC and/or PE are used simultaneously to label surface or intracellular antigens.

About This Assay

Cayman's 7-AAD Cell Viability Assay Kit employs 7-AAD as a fluorescent label for dead cells. A fixative/actinomycin D solution is included in the kit for cell fixation and blocking, making subsequent immunostaining of surface/intracellular antigens possible. This kit provides a convenient tool to quantify cytotoxic effects of environmental toxins or drug candidates.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

1. Assay Buffer Preparation

Dissolve each Cell-Based Assay Buffer Tablet (Item No. 10009322) with 100 ml of distilled water. Mix well to ensure that the tablet dissolves completely.

2. Staining Solution Preparation

Add 10 µl of Cell-Based Assay 7-AAD Staining Stock Solution (1,000X) (Item No. 400201) to 10 ml of Assay Buffer. Mix well.

Performing the Assay

1. Seed cells in a 6-, 12-, or 24-well plate at a density of 10^5-10^6 cells/well in 2, 1, or 0.5 ml of culture medium. Culture the cells in a CO_2 incubator at 37˚C for at least 24 hours before treatment.
2. The following day, treat cells with experimental compounds or vehicle for 24 hours, or for the period of time used in your typical experimental protocol.
3. At the end of the treatment, trypsinize (adherent cells) or collect cells (suspension cells). Centrifuge at 400 x g for five minutes to pellet the cells and wash once with 1 ml of Assay Buffer.
4. Resuspend cell pellet in Staining Solution (prepared on page 6) at a concentration of 1-5x10^6 cells/ml. It is important to achive a monodisperse cell suspension at this step by pipetting up and down repeatedly.
5. Incubate cells at room temperature for 10-15 minutes. Protect samples from light.
6. Centrifuge at 400 x g for five minutes to pellet the cells. Resuspend cell pellet in 0.5 ml of Assay Buffer. Pipette up and down repeatedly to achive a monodisperse cell suspension. Cells can now be analyzed in the FL3 channel of a flow cytometer with a 488 nm excitation laser. *Alternatively, if immunostaining for cell surface or intracellular antigens is desired, continue to step #7.*
7. Add a volume of 7-AAD Assay Fixative/Actinomycin D Solution (Item No. 400202) equal to the volume of Assay Buffer used in Step 6 to the cell suspension from step #6. Incubate in the dark at room temperature for 15-30 minutes.
8. Centrifuge at 400 x g for five minutes to pellet the cells. Wash twice with 0.5-1 ml of Assay Buffer.
9. Continue immunostaining according to your normal protocol.

PERFORMANCE CHARACTERISTICS

Flow Cytometry

An example of typical data obtained using flow cytometry is shown in the figure below. Your results may vary based on the cell type and experimental protocol used.

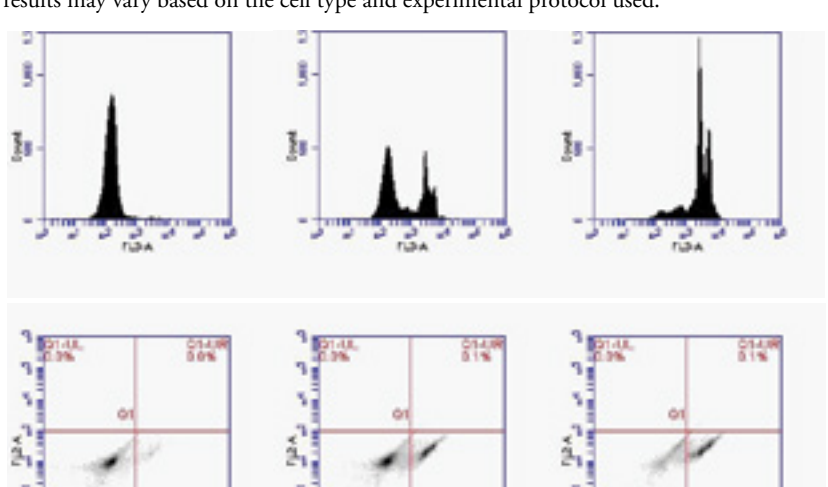


Figure 1: Effects of H_2O_2 on cell viability measured by 7-AAD. THP-1 cells were seeded in a 6-well plate at a density of 10^6 cells/well and cultured in a CO_2 incubator overnight. The next day, cells were treated with vehicle (upper and lower left panels), 0.05% (15 mM) H_2O_2 *(upper and lower middle panels)*, or 0.25% (75 mM) H_2O_2 *(upper and lower right panels)* for 24 hours at 37˚C in a CO_2 incubator. Cells were then pelleted and stained with Staining Solution according to the procedure described in this booklet. In the cells treated with vehicle (control), there were few dead cells *(1.3%, lower right quadrant of lower left panel)*. H_2O_2 at 0.05% caused death of 36.5% of the cells in a 24 hour period *(lower right quadrant of lower middle panel)*. When cells were treated with 0.25% H_2O_2 for 24 hours, most of cells were dead *(82.5%, lower right quadrant of lower right panel)*.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Poor signal	The amount of staining solution is not optimal	The amount of staining solution needed to obtain a good cytometric reading may need to be adjusted according to the cell type
High signal in untreated cells	A. Cells may not be healthy B. Cells may be fixed	A. Use only healthy, low passage cells B. Make sure that cells are not fixed before applying Staining Solution

References

1. Hynes, J., Floyd, S., Soini, A.E., *et al.* Fluorescence-based cell viability screening assays using water-soluble oxygen probes. *J. Biomol. Screen* **8**, 264-272 (2003).
2. Coder, D.M. Assessment of cell viability. *Current Protocols in Cytometry* **9.2.1-9.2.14** (1997).

Related Products

Apoptotic Blebs Assay Kit - Item No. 10010750
Caspase-3 Fluorescence Assay Kit - Item No. 10009135
Cell Cycle Phase Determination Kit - Item No. 10009349
LDH Cytotoxicity Assay Kit - Item No. 10008882
Lysosome/Cytotoxicity Dual Staining Kit - Item No. 600310
MTT Cell Proliferation Assay Kit- Item No. 10009365
Multi-Drug Resistance Assay Kit (Calcein AM) - Item No. 600370
Multi-Parameter Apoptosis Assay Kit - Item No. 600330
Phagocytosis Assay Kit (IgG PE) - Item No. 600540
20S Proteasome Assay Kit - Item No. 10008041
XTT Cell Proliferation Assay Kit - Item No. 10010200
For a complete list of related products please visit: www.caymanchem.com/catalog/10009856

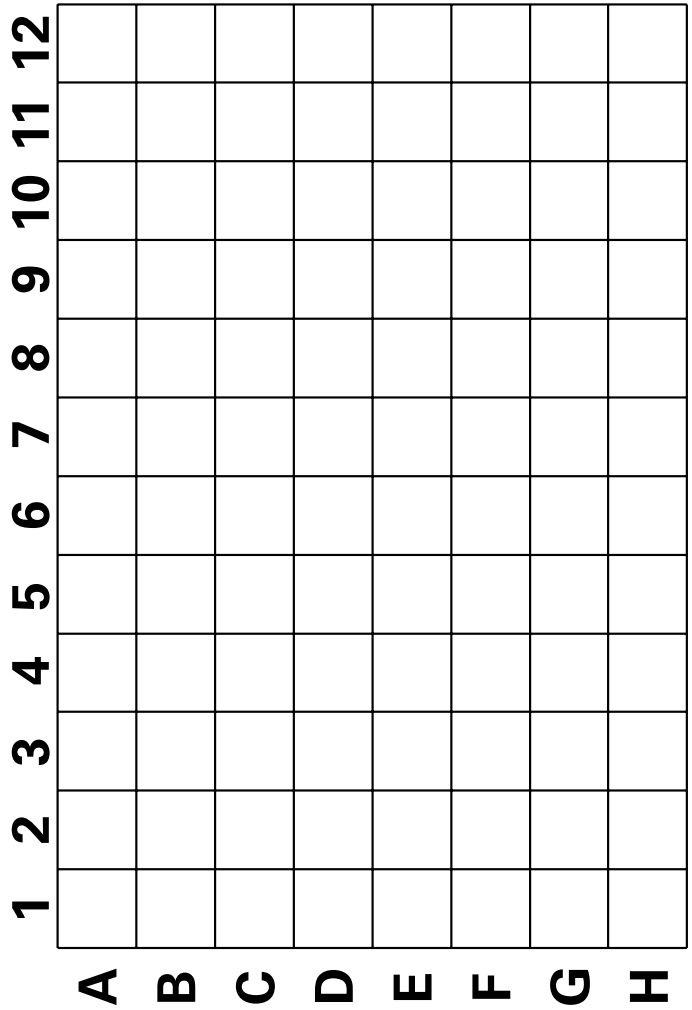
Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



NOTES