Lysosome/Cytotoxicity Dual Staining Kit

Item No. 600310

Customer Service 800.364.9897 * **Technical Support** 888.526.5351

www.caymanchem.com

TABLE OF CONTENTS

GENERAL INFORMATION
3 Materials Supplied
4 Precautions
4 If You Have Problems
4 Storage and Stability
4 Materials Needed but Not Supplied

INTRODUCTION
5 Background
5 About This Assay

PRE-ASSAY PREPARATION
6 Reagent Preparation

ASSAY PROTOCOL
7 Fluorescence Microscopy
8 Plate Reader Fluorescence Detection
9 Flow Cytometry

PERFORMANCE CHARACTERISTICS
10 Cell Staining
11 Plate Reader Fluorescence Detection
12 Flow Cytometry

RESOURCES
13 Troubleshooting
13 References
13 Related Products
14 Warranty and Limitation of Remedy
15 Plate Template
16 Notes

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	Quantity/Size	Storage
600311	NBD-PZ Stock Solution	1 vial/100 µl	-20°C
10009322	Cell-Based Assay Buffer Tablet	1 vial/5 tablets	Room Temperature
10011234	Cell-Based Propidium Iodide Solution	1 vial/250 µl	4°C
10012670	Chloroquine Positive Control (25 mM)	1 vial/50 µl	4°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 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

E-Mail: 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-, 24-, or 96-well cell culture plates
2. HepG2 cells (can be obtained from ATCC); other cell lines can also be used
3. A fluorescence microscope or plate reader capable of detecting NBD-PZ at excitation and emission wavelengths of 485 and 535 nm, respectively, and propidium iodide at excitation and emission wavelengths of 536 and 617 nm, respectively
4. Adjustable pipettes and a repeating pipettor

INTRODUCTION

Background

Lysosomes are single membrane-bound acidic intracellular compartments that break down cellular waste products, fats, carbohydrates, proteins, and other macromolecules into simple compounds. Normal lysosome function enables cells to efficiently mobilize and recycle cellular constituents, but also prevents the accumulation of damaged organelles, misfolded proteins, and invading microorganisms.[1] Although lysosomes are found in all animal cells, they are most abundant in disease-fighting cells such as leukocytes. Lysosomes contain about 50 different degradative enzymes, which are optimally active at the acidic pH (about 5) that is maintained within lysosomes but not at the neutral pH (about 7.2) characteristic of the rest of the cytoplasm.[2] These enzymes work together to control protein and organelle homeostasis. Disorders of these lysosomal enzymes cause several human diseases including Tay-Sachs disease, arthritis, and neurodegenerative diseases. Evidence shows that in Parkinson's disease brains, expression of lysosomal enzymes, such as cathepsin D, is significantly decreased. Upregulation of lysosomal genes is beginning to be explored as a potential therapeutic approach against neurodegenerative diseases such as Parkinson's Disease.[3]

About This Assay

Cayman's Lysosome/Cytotoxicity Dual Staining Kit provides a convenient tool for studying lysosome function at the cellular level. The kit employs 4-nitro-7-(1-piperazinyl)-2,1,3-benzoxadiazole (NBD-PZ), which is membrane permeable and reacts with carboxylic acids in the acidic luminal environment of lysosomes,[4] as a probe for the detection of lysosomes in cultured cells. Propidium iodide is used as a marker of cell death. Chloroquine, a known inhibitor of lysosome function, is included as a positive control. The kit provides sufficient reagents to effectively treat/stain 960 individual wells of cells when utilized in a 96-well plate format. Lower density plates will still require approximately the same amount of reagents on a per plate basis. Therefore, up to 10 plates worth of cells can be examined irrespective of the number of wells/plate. Exceptions include protocols in which non-adherent cells are utilized and flow cytometry detection methods are employed.

PRE-ASSAY PREPARATION

Reagent Preparation

1. Cell-Based Assay Buffer Preparation

Dissolve each Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of distilled water. This Buffer should be stable for approximately one year at room temperature.

2. NBD-PZ/Propidium Iodide Dual Staining Solution Preparation

To 10 ml of culture medium used for your cells, add 10 µl of NBD-PZ Stock Solution (Item No. 600311) and 20 µl of Cell-Based Propidium Iodide Solution (Item No. 10011234). Mix well. Protect from light.

General Precautions

- NBD-PZ is extremely light sensitive and is photobleached quickly. 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 7-9, it is imperative that samples be analyzed immediately following completion of the staining. It is recommended that NBD-PZ staining be measured first and the propidium iodide staining be measured second to avoid depletion of the NBD-PZ fluorescence during analysis.

ASSAY PROTOCOL

Fluorescence Microscopy

The following protocol is for 96-well plates. The volume of Buffer and Staining Solution should be adjusted accordingly if you are using a different plate density.

1. Seed a 96-well plate with 5 x 10^4 cells/well. Grow cells overnight.
2. The next day, treat the cells with experimental compounds or vehicle control for 24-72 hours, or for the period of time used in your typical experimental protocol. To use the Chloroquine Positive Control (Item No. 10012670), dilute 1:1,000-1:5,000 into your culture medium.
3. After the treatment period, centrifuge the plate for five minutes at 400 x g at room temperature.
4. Aspirate the supernatant.
5. Add 100 µl of the NBD-PZ/Propidium Iodide Dual Staining Solution to each well. Be careful to not disturb the cell layer. Incubate the cells for 10 minutes in a cell culture incubator at 37°C.
6. Centrifuge the plate for five minutes at 400 x g at room temperature.
7. Aspirate the supernatant.
8. Add 100 µl of Cell-Based Assay Buffer to each well. Be careful to not disturb the cell layer.
9. The cells are now ready for analysis by fluorescent microscopy and must be analyzed immediately. Lysosomes stained by NBD-PZ can be detected with a filter capable of excitation and emission at 485 nm and 535 nm, respectively. Dead cells are stained by propidium iodide and can be detected with filters usually designed to detect rhodamine (excitation/emission = 540/570 nm, respectively) or Texas Red (excitation/emission = 590/610 nm, respectively).

Plate Reader Fluorescence Detection

The following protocol is designed for a 96-well plate. Adjust volumes accordingly for other plate densities. A 96-well black culture plate should be used for this method. Optimal conditions will depend on the cell type being used.

1. Seed a 96-well black culture plate with 5 x 10^4 cells/well. Grow cells overnight.
2. The next day, treat the cells with experimental compounds or vehicle control for 24-72 hours, or for the period of time used in your typical experimental protocol. To use the Chloroquine Positive Control (Item No. 10012670), dilute 1:1,000-1:5,000 into your culture medium.

   NOTE: Differences in cell density can significantly affect results. Ensure that experimental compounds do not significantly inhibit cell proliferation.
3. After the treatment period, centrifuge the plate for five minutes at 400 x g at room temperature.
4. Aspirate the supernatant.
5. Add 100 µl of the NBD-PZ/Propidium Iodide Dual Staining Solution to each well. Be careful to not disturb the cell layer. Incubate the cells for 10 minutes in a cell culture incubator at 37°C.
6. Centrifuge the plate for five minutes at 400 x g at room temperature.
7. Aspirate the supernatant.
8. Add 100 µl of Cell-Based Assay Buffer to each well. Be careful to not disturb the cell layer.
9. The cells are now ready for analysis in a plate reader and must be analyzed immediately. Lysosome staining intensity can be detected by using an excitation wavelength of 485 nm and an emission wavelength of 535 nm. The degree of cell death can be assessed by measuring propidium iodide staining intensity at excitation and emission wavelengths of 536 and 617 nm, respectively.

Flow Cytometry

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. Grow cells overnight.
2. The next day, treat the cells with experimental compounds or vehicle control for 24-72 hours, or for the period of time used in your typical experimental protocol. To use the Chloroquine Positive Control (Item No. 10012670), dilute 1:500-1:10,000 into your culture medium.
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.
4. Aspirate supernatant.
5. Resuspend cell pellet in 0.5 to 1 ml of NBD-PZ/Propidium Iodide Dual Staining Solution. It is important to achieve a monodisperse cell suspension at this step by pipetting up and down repeatedly. Incubate the cells for 10 minutes in a cell culture incubator at 37°C.
6. Centrifuge at 400 x g for five minutes to pellet the cells.
7. Resuspend cell pellet in 0.5-1.0 ml of Assay Buffer depending on cell number. Pipette up and down repeatedly to achieve a monodisperse cell suspension.
8. The cells are now ready for analysis by a flow cytometer and must be anyalyzed immediately. Lysosomes staining can be detected in the FL1 channel and dead cells stained by propidium iodide can be detected in the FL2 channel.

PERFORMANCE CHARACTERISTICS

Cell Staining

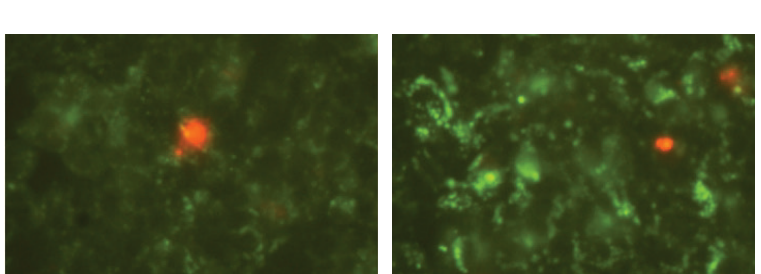


Figure 1. Chloroquine increases lysosome accumulation but not cell death in HepG2 cells as measured by fluorescent microscopy. HepG2 cells were seeded at a density of 5 x 10^4 cells/well and incubated overnight at 37°C. The next day, cells were treated with either vehicle or different concentrations of chloroquine. On the third day, cells were processed for NBD-PZ and propidium iodide staining according to the protocol described above. *Panel A:* HepG2 cells treated with vehicle. There was a basal level of lysosome staining, indicated by faint green staining of NBD-PZ. Few dead cells were detected (red nuclei staining by propidium iodide). *Panel B:* NBD-PZ and propidium iodide staining of HepG2 cells treated with 12.5 µM chloroquine. Note the increase in NBD-PZ fluorescence intensity but not the numbers of propidium iodide positive dead cells compared to the cells treated with vehicle.

Plate Reader Fluorescence Detection

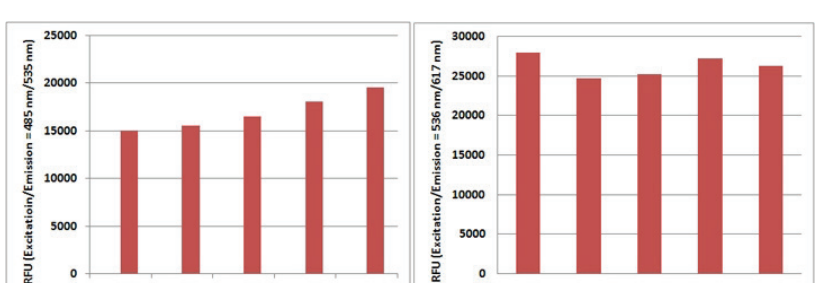


Figure 2. Chloroquine increases lysosome accumulation but not cell death in HepG2 cells. HepG2 cells were seeded in a 96-well plate at a density of 5 x 10^4 cells/well and incubated overnight at 37°C. The next day, cells were treated with different concentrations of chloroquine overnight. On the third day, cells were stained with NBD-PZ/Propidium Iodide Dual Staining Solution according to the protocol described above. *Left panel:* Chloroquine treatment increased NBD-PZ (lysosome staining) fluorescence intensity, indicating that chloroquine caused an increase in lysosome accumulation in HepG2 cells. *Right panel:* Chloroquine treatment did not cause an increase in propidium iodide staining, indicating that at the concentrations used here, chloroquine did not cause cytotoxicity in HepG2 cells.

Flow Cytometry

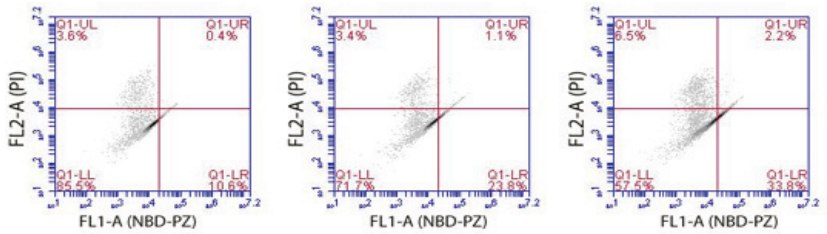


Figure 3. Chloroquine increases lysosome accumulation and cytotoxicity in Jurkat cells. Jurkat cells were seeded in a 6-well plate at a density of 5 x 10^5 cells/well in RPMI culture medium and incubated overnight at 37°C. The next day, cells were treated with different concentrations of chloroquine overnight. On the third day, cells were stained with NBD-PZ/Propidium Iodide Dual Staining Solution according to the protocol described above. *Left panel:* Cells treated with vehicle showed a low percentage of cell death (4%-upper left and upper right quadrants) and about 11% of cells had detectable lysosome staining (lower right and upper right quadrants). *Middle panel:* When cells were treated with 12.5 µM chloroquine there was not an increase in the percentage of cell death (4.5%-upper left and upper right quadrants) but there was an increase in detectable lysosome staining (24.9%-lower right and upper right quadrants). *Right panel:* 50 µM chloroquine treatment caused a significant increase in both cell death (8.7%-upper left and upper right quadrants) and lysosome accumulation (36%-lower right and upper right quadrants).

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Low NBD-PZ staining in all treatments, including positive control	A. Cells are not healthy B. Cells have a low number of lysosomes and do not respond to treatment	A. Use only healthy cells B. Use a different cell type
High level of propidium iodide staining, including in control cells	Cells are not healthy or are dead	Use only healthy living cells
Low fluorescence intensity in both NBD-PZ and propidium iodide staining	A. Cell density too low B. Cells are lost during processing	A. Increase cell density B. Gently aspirate supernatant to ensure most of the cells stay on the plate

References

1. Fehrenbacher, N. and Jäättelä, M. Lysosomes as targets for cancer therapy. *Cancer Res.* **65(8)**, 2993-2995 (2005).
2. Zhang, L., Sheng, R., and Qin, Z. The lysosome and neurodegenerative diseases. *Acta Biochim. Biophys. Sin.* **41(6)**, 437-445 (2009).
3. Schneider, L. and Zhang, J. Lysosomal function in macromolecular homeostasis and bioenergetics in Parkinson's disease. *Mol. Neurodegener.* **5**, 14 (2010).
4. Ishiguro, K., Ando, T., and Goto, H. Novel application of 4-nitro-7-(1-piperazinyl)-2,1,3-benzoxadiazole to visualize lysosomes in live cells. *Biotechniques* **45(4)**, 465-468 (2008).

Related Products

7-AAD Cell Viability Assay Kit - Item No. 10009856

Related Products cont.

7-AAD/CFSE Cell-Mediated Cytotoxicity Assay Kit - Item No. 600120
Apoptotic Blebs Assay Kit - Item No. 10010750
Autophagy/Cytotoxicity Dual Staining Kit - Item No. 600140
Caspase-3 Fluorescence Assay Kit - Item No. 10009135
Cell Cycle Phase Determination Kit - Item No. 10009349
CFSE Cell Division Assay Kit - Item No. 10009853
Glutathione Cell-Based Detection Kit (Blue Fluorescence) - Item No. 600360
LDH Cytotoxicity Assay Kit - Item No. 10008882
MTT Cell Proliferation Assay Kit - Item No. 10009365
20S Proteasome Assay Kit - Item No. 10008041
WST-1 Cell Proliferation Assay Kit - Item No. 10008883
WST-8 Cell Proliferation Assay Kit - Item No. 10010199
XTT Cell Proliferation Assay Kit - Item No. 10010200

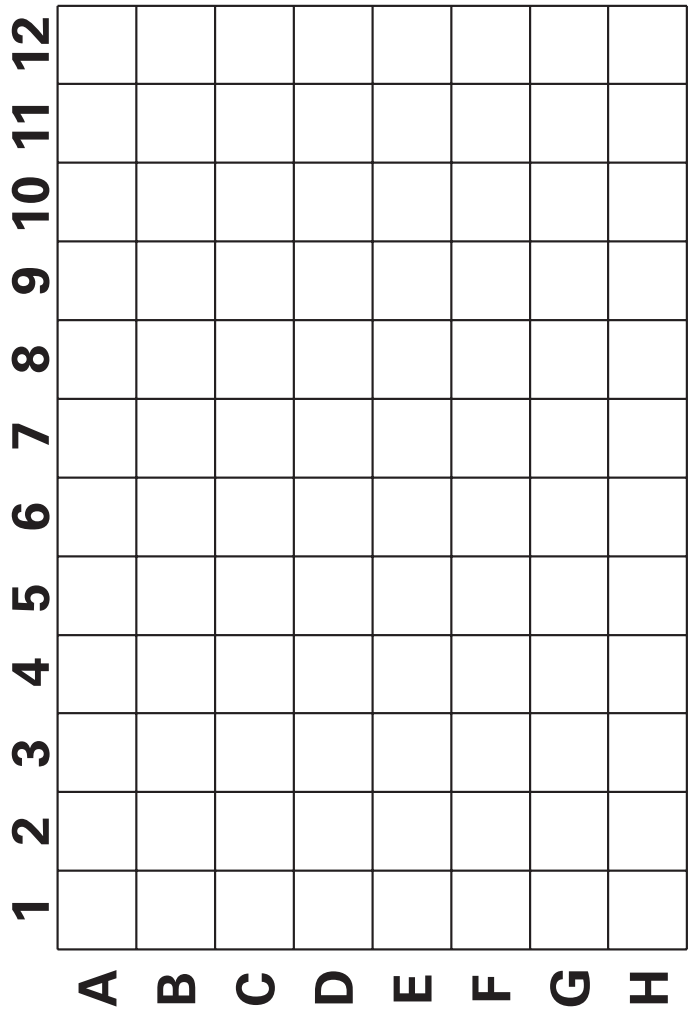
Warranty and Limitation of Remedy

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