Lysosomal Staining Kit (Red Fluorescence)

Item No. 601810

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

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. After opening, remove the components and stored as stated below.

Item Number	Item	Quantity/Size	Storage
701785	Lysosomal Staining Assay Buffer	1 vial/50 ml	-20°C
701784	Lysosomal Staining Reagent (Red Fluorescence)	1 vial/20 µl	-20°C
701782	Bafilomycin Control Reagent	1 vial/20 µl	-20°C
600332	Cell-Based Assay Hoechst Dye	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 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

Fax: 734-971-3640

E-Mail: 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. Black 96-well tissue culture plates
2. A microscope or imager equipped with a Texas Red filter set and an optional DAPI filter set

INTRODUCTION

About This Assay

Lysosomes are intracellular organelles that contain enzymes used for the hydrolysis of waste materials and other cellular debris. The pH of the lysosome is ~4.5-4.8 and is optimal for these hydrolytic enzymes. The lysosomal pH gradient is maintained through vacuolar ATPases which pump protons into the lysosomes.

Cayman's Lysosomal Staining Kit (Red Fluorescence) utilizes a red fluorescent dye that permeates the lysosomes based on pH gradient. Once protonated, the dye is unable to leave the lysosome resulting in enhanced fluorescence. Included in this kit is bafilomycin A_1, which is an inhibitor of the vacuolar ATPase. Treatment with bafilomycin A_1 results in decreased lysosomal fluorescence upon staining with the lysosomal staining reagent.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Lysosomal Staining Assay Buffer – (Item No. 701785)**

   This bottle contains 50 ml of Lysosomal Staining Assay Buffer and is ready to use as supplied. Unused reagent can be stored at -20°C for up to 6 months.

2. **Lysosomal Staining Reagent (Red Fluorescence) – (Item No.701784)**

   This vial contains 20 µl of Lysosomal Staining Reagent (Red Fluorescence) and is ready to use as supplied. Unused reagent can be aliquoted and stored at -20°C until the expiration date of the kit. Avoid multiple freeze thaw cycles.

3. **Bafilomycin Control - (Item No. 701782)**

   This vial contains 20 µl of a 100X Bafilomycin Control Reagent and is ready to use as supplied. Unused reagent can be stored at -20°C until the expiration date of the kit. Avoid multiple freeze thaw cycles.

4. **Cell-Based Assay Hoechst Dye (Item No. 600332)**

   This vial contains a 20 mM solution of Hoechst Dye in water and is ready to use as supplied. Store unused portion of this reagent at 4°C until the expiration date of the kit. Cell-Based Assay Hoechst Dye is included as an optional reagent for nuclear counter-staining that can be useful for cell counting/normalization.

Pre-Assay Preparation

1. **Lysosomal Staining Solution**

   Dilute Lysosomal Staining Reagent 1:1,000 by adding 13 µl of Lysosomal Staining Reagent to 13 ml of room temperature Lysosomal Staining Assay Buffer. To add a nuclear counterstain, add 6 µl of Cell-Based Assay Hoechst Dye to the 13 ml of Lysosomal Staining Assay Buffer that contains the Lysosomal Staining Reagent.

   This amount of Lysosomal Staining Reagent will stain one 96-well plate. If less volume is required, Lysosomal Staining Solution can be scaled accordingly. Lysosomal Staining Solution should be protected from light and discarded after use.

   NOTE: The above protocol is for a suggested concentration for lysosomal staining. We recommend performing a cell and dye titration before starting, when working with unfamiliar cell lines.

2. **Bafilomycin Control Working Reagent (10X)**

   Prepare the Bafilomycin Working Control Reagent by diluting Bafilomycin Control 1:10 in Cell Based Assay Buffer. Discard any unused Bafilomycin Control Working Reagent after use.

ASSAY PROTOCOL

For Adherent Cells

This protocol is written for 96-well plates. Volumes can be adjusted to suit different plate/vessel types. We recommend diluting experimental compounds in Lysosomal Staining Assay Buffer in a 10X stock. This may vary based on the solubility of experimental compounds. If longer incubations are required, experimental compounds can be added 6 hours after plating cells.

1. Plate cells at a desired concentration in a black, tissue culture treated 96-well plate and culture cells, per desired protocol, in the media best suited for your cell line. Ensure that cells are healthy and not overgrown.

   NOTE: If working with an unknown cell line, we recommend performing a cell seeding titration to determine the optimal seeding density.

2. Ensure that a minimum of 3 wells (3 replicates) are designated as bafilomycin control wells. Remaining wells can be used as sample or control wells based on your experimental design. We recommend performing each condition in triplicate.
3. Remove spent culture media, and replace with 90 µl/well of fresh culture media.
4. Add 10 µl of Bafilomycin Control Working Reagent to wells designated as bafilomycin controls.
5. Add 10 µl of experimental compounds or Lysosomal Staining Assay Buffer to remaining wells.
6. Incubate plate for 1 hour at 37°C.
7. Carefully remove culture media and replace with 100 µl/well of Lysosomal Staining Solution.
8. Incubate plate for 30 minutes at 37°C.
9. Carefully remove Lysosomal Staining Solution and replace with 100 µl/well of Lysosomal Staining Assay Buffer.
10. Carefully remove the Lysosomal Staining Assay Buffer and replace with an additional 100 µl/well of Lysosomal Staining Assay Buffer.
11. Plate can be imaged using a microscope or imager equipped with a Texas Red and DAPI filter. The excitation and emission maxima for the Lysosomal Staining Reagent and Cell-Based Assay Hoechst Dye are 575/595 nm and 350/450 nm, respectively.

ANALYSIS

Performance Characteristics

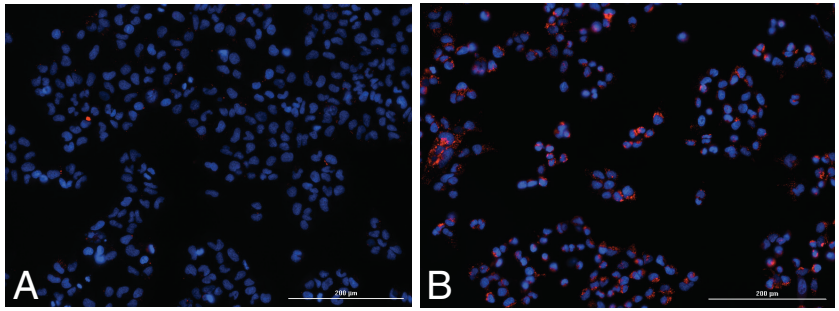


Figure 1. *Panel A* shows Huh7 cells treated with 1 µM Bafilomycin A_1 *versus Panel B* shows Huh7 cells treated with vehicle. Cell images were obtained using the Cytation™ 5 Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc.).

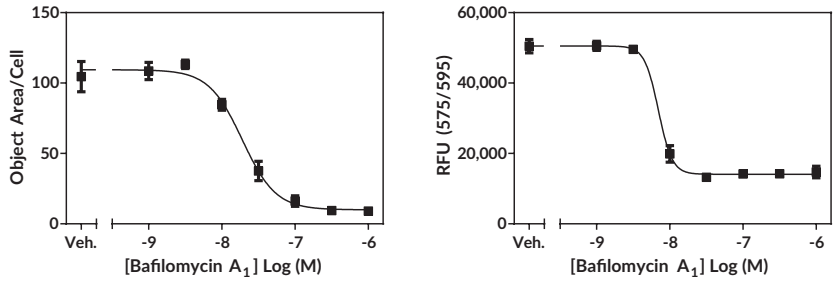
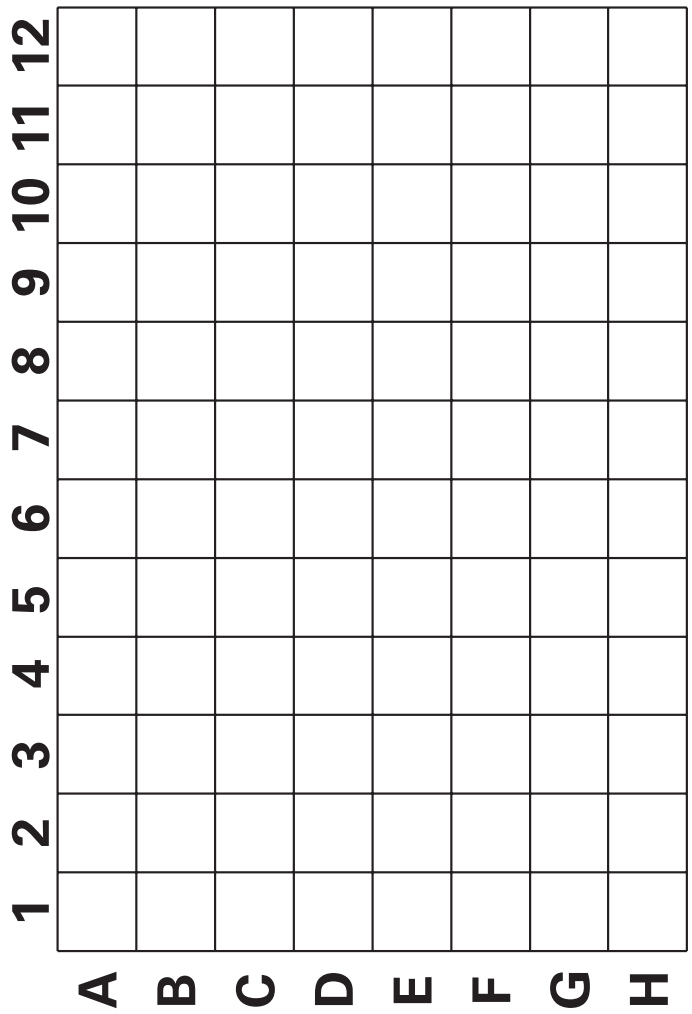


Figure 2. Concentration response of bafilomycin A_1 on lysosomal fluorescence analyzed using object area/cell or fluorescence intensity using the Lysosomal Staining Kit.

RESOURCES

Troubleshooting

Problem	Recommended Solutions
No staining	A. Perform Cell/Dye titration to optimize staining conditions B. Adjust gain/exposure time
Overstaining	A. Perform Cell/Dye titration to optimize staining conditions B. Adjust gain/exposure time



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.