



Autophagy Modulation Reagent Kit

Item No. 44898

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TABLE OF CONTENTS

GENERAL INFORMATION	3	Materials Supplied
	3	Safety Data
	4	Precautions
	4	If You Have Problems
	5	Storage and Stability
	5	Materials Needed but Not Supplied
INTRODUCTION	6	Description
PREPARATION	7	Reagent Preparation
RESOURCES	9	References
	10	Notes
	11	Warranty and Limitation of Remedy

GENERAL INFORMATION

Materials Supplied

Item No.	Item Name	Quantity/Size	Storage
10997	Torin 1	1 vial/1 mg	-20°C
16979	SAR405	1 vial/1 mg	-20°C
11038	Bafilomycin A ₁	1 vial/1 mg	-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 (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Cell culture-grade DMSO

Description

The components of this kit enable induction and/or inhibition of autophagy in cultured cells.¹⁻⁴ Each component in this kit is provided separately, and instructions are included to reconstitute these reagents for direct dilution into cell culture media. Some reagents may be provided in excess and may be properly disposed of after cell media preparation. Prior to use, reagents must be stored as indicated on page 3.

Torin 1 is a selective inhibitor of mTORC1 and mTORC2, key negative regulators of autophagy.^{1,2} Inhibition of mTORC1 by torin 1 relieves suppression of the Unc-51-like autophagy activating kinase 1 (ULK1) complex activity and promotes initiation of autophagy.⁵

SAR405 is an inhibitor of the PI3K class III isoform vacuolar protein sorting 34 (VPS34), an essential kinase in autophagosome nucleation.^{1,3} By inhibiting VPS34 activity, SAR405 suppresses autophagosome formation and blocks autophagy at an early stage.

Bafilomycin A₁ is an inhibitor of vacuolar H⁺-ATPases (V-ATPases), preventing the acidification of lysosomes.^{1,4} This in turn blocks the fusion of autophagosome with lysosomes. Bafilomycin A₁ is used to distinguish defects in autophagosome formation *versus* autophagic flux.

Reagent Preparation

General Guidelines

- Prepare each reagent according to the instructions below prior to use in cell-based experiments.
- Concentrated stock solutions may be stored at -20°C or below, protected from light for up to six months.
- Prepare single-use aliquots to minimize freeze-thaw cycles.
- Final concentrations of solvents in cell culture medium should be kept at ≤0.1% (v/v), preferably ≤0.05%.
- Recommended final concentrations are provided as general guidelines. Actual performance may depend on cell type, density, media formulation, and experimental design. Users should perform an optimization experiment to determine the appropriate concentration for each reagent.

Preparation of Individual Stock Solutions

1. Prepare a 10 mM concentrated stock solution of torin 1 by adding 165 μ l of sterile DMSO to the vial and gently mix until fully dissolved. Prepare a 0.5 mM intermediate stock solution by diluting the 10 mM concentrated stock solution 20-fold in DMSO. *NOTE: It is not necessary to prepare the full volume of intermediate stock solution at once; generate only the volume needed for upcoming experiments to avoid excessive aliquots.* This 0.5 mM torin 1 solution may be diluted in cell culture media to a final concentration of 50-200 nM.
2. Prepare a 2 mM stock solution of SAR405 by adding 1,126 μ l of sterile DMSO to the vial and gently mix until fully dissolved. This 2 mM SAR405 solution may be diluted in cell culture media to a final concentration of 100-1,000 nM.
3. Prepare a 10 mM concentrated stock solution of bafilomycin A₁ by adding 161 μ l of sterile DMSO to the vial and gently mix until fully dissolved. Prepare a 0.5 mM intermediate stock solution by diluting the 10 mM concentrated stock solution 20-fold in DMSO. *NOTE: It is not necessary to prepare the full volume of intermediate stock solution at once; generate only the volume needed for upcoming experiments to avoid excessive aliquots.* This 0.5 mM bafilomycin A₁ solution may be diluted in cell culture media to a final concentration of 10-100 nM.

RESOURCES

References

1. Klionsky, D. J., Abdalla, F. C., Abeliovich, H., *et al.* Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy* **8**(4), 445-544 (2012).
2. Kim, Y.C. and Guan, K.-L. mTOR: A pharmacological target for autophagy regulation. *J. Clin. Invest.* **125**(1), 25-32 (2015).
3. Ronan, B., Flamand, O., Vescovi, L., *et al.* A highly potent and selective Vps34 inhibitor alters vesicle trafficking and autophagy. *Nat. Chem. Biol.* **10**(2), 1013-1019 (2014).
4. Yamamoto, A., Tagawa, Y., Yoshimori, T., *et al.* Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells. *Cell Struct. Funct.* **23**(1), 33-42 (1998).
5. Thoreen, C.C., Kang, S.A., Chang, J.W., *et al.* An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *J. Biol. Chem.* **284**(12), 8023-8032 (2009).

Warranty and Limitation of Remedy

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