ER Stress/Unfolded Protein Response (UPR) Reagent Kit

Item No. 44899

www.caymanchem.com
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

Item No.	Item Name	Quantity/Size	Storage
10522	Thapsigargin	1 vial/1 mg	-20°C
11445	Tunicamycin Mixture	1 vial/1 mg	-20°C
9003379	Tauroursodeoxycholic Acid (sodium salt hydrate)	1 vial/25 mg	-20°C
14735	Salubrinal	1 vial/1 mg	-20°C
19151	KIRA6	1 vial/1 mg	-20°C
36113	Ceapin-A7	1 vial/5 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
2. Cell cultured medium

INTRODUCTION

Background

Endoplasmic reticulum (ER) stress is an imbalance in ER protein-folding demand and capacity, leading to the accumulation of misfolded proteins.[1,2] Misfolded proteins are targeted for ER-associated degradation (ERAD), but when they continue to accumulate, the unfolded protein response (UPR) is triggered.[1] Initially, UPR signaling halts translation *via* protein kinase R-like ER kinase (PERK) signaling to reduce the protein-folding load and induces the expression of chemical chaperones *via* inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6) to enhance protein folding quality. If ER homeostasis is not restored, PERK and IRE1 signaling lead to the induction of apoptosis.[1,2] ER stress is associated with a variety of disease states, including cardiovascular, autoimmune, and neurodegenerative diseases.[1]

Description

The components of Cayman's ER Stress/UPR Reagent Kit enable the induction and selective modulation of ER stress and the UPR in cultured cells. Each reagent is supplied individually, allowing users to strategically combine inducers and modulators, as described below, to interrogate specific UPR branches (PERK, IRE1, and/or ATF6).

- Thapsigargin inhibits sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), causing depletion of calcium ions in the ER and impairment in chaperone function.[3] This induces ER stress and robust UPR activation across PERK, IRE1, and ATF6 pathways.
- Tunicamycin blocks N-linked glycosylation in the ER by inhibiting N-acetylglucosamine-1-phosphotransferase, leading to the accumulation of misfolded proteins and UPR activation through the PERK, IRE1, and ATF6 pathways.[4]
- Tauroursodeoxycholic acid (TUDCA) acts as a chemical chaperone that stabilizes protein folding in the ER, thereby reducing ER stress.[5,6] It has commonly been used as a protective agent to broadly suppress UPR activation and prevent ER stress-mediated cell death.[7]
- Salubrinal inhibits the growth arrest and DNA damage-inducible protein 34-protein phosphatase 1 (GADD34-PP1) complex, preventing dephosphorylation of the PERK target eukaryotic translation initiation factor 2α (eIF2α).[8] Phosphorylation of eIF2α by PERK leads to prolonged attenuation of protein translation and helps reduce ER protein-folding stress.[1] Salubrinal can be used to evaluate the contribution of PERK signaling to the ER stress response.
- KIRA6 is a selective inhibitor of IRE1α kinase activity that indirectly suppresses its RNase function.[9] By blocking IRE1α signaling, KIRA6 reduces the IRE1-mediated UPR. KIRA6 can be used to evaluate the contribution of IRE1 signaling in ER stress.
- Ceapin-A7 blocks the trafficking of ATF6 from the ER to the Golgi and thus inhibits its activation.[10] Ceapin-A7 can be used to evaluate the role of ATF6 in ER stress.

Instructions are included to reconstitute these reagents for direct dilution into cell culture media. Some reagents may be provided in excess and can be properly disposed of after cell media preparation.

Reagent	Primary Effect	Effect on ER Stress		
		PERK Axis	IRE1 Axis	ATF6 Axis
Thapsigargin	ER Ca^{2+} depletion	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑
Tunicamycin	Misfolded protein accumulation	↑ ↑	↑ ↑	↑
TUDCA	Stabilizes protein folding	↓ ↓	↓ ↓	↓ ↓
Salubrinal	Sustained eIF2α phosphorylation; reduces protein translation	↓ ↓	--	--
KIRA6	IRE1 inhibition	--	↓ ↓ ↓	--
Ceapin-A7	ATF6 inhibition	--	--	↓ ↓ ↓

Table 1. Summary of the included reagents' effects on ER stress in the three major branches of the UPR

PREPARATION

Reagent Preparation

General Guidelines

- Prepare each reagent according to the instructions (see page 9) 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.
- Intermediate stock solutions in cell culture medium should be prepared just prior to use and discarded within a day. *NOTE: When preparing intermediate stock solutions, it is not necessary to dilute the entire concentrated stock solution all at once; generate only the volume needed for upcoming experiments.*
- 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, cell 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 thapsigargin by adding 154 µl of sterile DMSO to the vial and gently mixing until fully dissolved. Prepare a 1 mM intermediate stock solution by diluting the 10 mM concentrated stock solution 10-fold in DMSO. This 1 mM thapsigargin solution may be diluted in cell culture media to a final concentration of 50-500 nM.
2. Prepare a 5 mg/ml concentrated stock solution of tunicamycin mixture by adding 200 µl of sterile DMSO to the vial and gently mixing until fully dissolved. Prepare a 10 µg/ml intermediate stock solution by diluting the 5 mg/ml concentrated stock solution 500-fold in cell culture medium. This 10 µg/ml solution may be further diluted in cell culture media to a final concentration of 0.1-1 µg/ml.
3. Prepare a 2 mM stock solution of TUDCA by dissolving 2 mg in 2 ml of cell culture medium and gently mixing until fully dissolved. The stock solution may be further diluted into cell culture medium to a final concentration of 100-1,000 µM. Use the TUDCA aqueous stock solution within one day.
4. Prepare a 5 mM concentrated stock solution of salubrinal by adding 417 µl of sterile DMSO to the vial and gently mixing until fully dissolved. This salubrinal solution may be diluted in cell culture media to a final concentration of 1-50 µM.
5. Prepare a 10 mM concentrated stock solution of KIRA6 by adding 193 µl of sterile DMSO to the vial and gently mixing until fully dissolved. Prepare a 50 µM intermediate stock solution by diluting the 10 mM concentrated stock solution 200-fold in cell culture medium. This 50 µM KIRA6 solution may be diluted in cell culture media to a final concentration of 0.5-5 µM.
6. Prepare a 20 mM concentrated stock solution of ceapin-A7 by adding 532 µl of sterile DMSO to the vial and gently mixing until fully dissolved. Prepare a 200 µM intermediate stock solution by diluting the 20 mM concentrated stock solution 100-fold in cell culture medium. This ceapin-A7 solution may be diluted in cell culture media to a final concentration of 2-20 µM.

References

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10. Gallagher, C.M. and Walter, P. *eLife* **5**, e11880 (2016).

NOTES

Warranty and Limitation of Remedy

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