Rapamycin

mTOR inhibitor

Catalog code: tlrl-rap

https://www.invivogen.com/rapamycin

For research use only

Version 22I20-MM

PRODUCT INFORMATION

Contents

- 5 mg Rapamycin

Storage and stability

- Rapamycin is provided as a solid and shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots of Rapamycin and store at -20 °C. Avoid repeated freeze-thaw cycles. Resuspended product is stable for 3 months when properly stored.

Quality control

- Purity ≥95% (UHPLC)
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) is confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Rapamycin is an inhibitor of mTOR (mammalian target of rapamycin), which regulates cell growth and metabolism in response to environmental cues. Rapamycin is also an inducer of autophagy, as inhibition of mTOR mimics cellular starvation by blocking signals required for cell growth and proliferation[1]. Specifically, it simultaneously binds to the intracellular receptor protein FKBP12 and to the FKBP12/rapamycin-binding (FRB) domain of mTOR. This results in the acute and direct inhibition of mTORc1 signaling. While mTORc1 inhibition by Rapamycin is well established, its effect on mTORc2 is less well characterized. Previously, the mTORc2 complex was thought to be Rapamycin-insensitive. However, studies show that prolonged Rapamycin treatment inhibits the assembly and function of mTORc2 in some, but not all, cell lines or tissues[2, 3].

1. Jung CH. *et al.*, 2010. mTOR regulation of autophagy. FEBS Lett. 584:1287-95. **2. Sarbassov D.D. *et al.*, 2006.** Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell. 22:159-68. **3. Schreiber K.H. *et al.*, 2015.** Rapamycin-mediated mTORC2 inhibition is determined by the relative expression of FK506-binding proteins. Aging Cell.14:265-73. **4. Decuypere J.P. et al., 2013.** mTOR-Controlled Autophagy Requires Intracellular $Ca^{(2+)}$ Signaling. PLoS One. 8:e61020. **5. Li Y. *et al.*, 2016.** Rapamycin-induced autophagy sensitizes A549 cells to radiation associated with DNA damage repair inhibition. Thorac Cancer. 7:379-86.

CHEMICAL PROPERTIES

CAS number: 53123-88-9

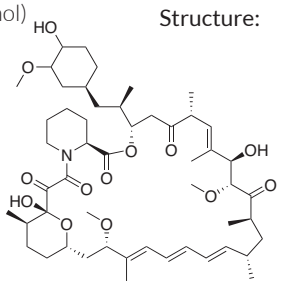
Synonym: Antibiotic AY 22989; Sirolimus

Solubility: 10 mM (DMSO), 2mM (ethanol)

Molecular weight: 914.17 g/mol

Formula: $C_{51}H_{79}NO_{13}$

Structure:



METHODS

Preparation of 10 mM (stock solution

1. Add 550 µl DMSO to 5 mg Rapamycin and vortex until solubilized.
2. Prepare aliquots of Rapamycin and store at -20 °C.
3. Once solubilized, dilutions can be prepared by adding sterile water.

Working concentrations: 100 nM-25 µM

Autophagy reporter assay:

Described below is a protocol to study the effects of rapamycin in HeLa-Difluo™ hLC3b cells, an autophagy reporter cell line derived from the human epithelial carcinoma HeLa cell line. These cells express two fluorescent reporter genes (RFP and GFP) fused to the N-terminal of the LC3 protein. The expression of this fusion protein enables the monitoring of autophagic flux in real time. For more information, visit www.invivogen.com/hela-difluo-hlc3.

Day 1

1. Prepare a HeLa-Difluo™ hLC3b cell suspension at ~100,000 cells/ml.
2. Add 500 µl of cell suspension per well of a 24-well plate.
3. Leave to incubate overnight at 37 °C in a 5% CO_2 incubator.

Day 2

1. Remove test medium and rinse cells with pre-warmed, sterile PBS.
2. Add 450 µl of test medium to every well of a 24-well plate.
3. Add 50 µl of Rapamycin at a final concentration of 25 µM per well.

Note: We recommend including a negative control such as sterile PBS.

4. Incubate at 37 °C.
5. Monitor the autophagic flux at different time intervals (e.g. after 30 min, 1h, 2h30 and 6h) using a high-resolution fluorescent microscope.

PROTOCOLS

For reference only; as described in the indicated publications.

Cell Culture Assay[4]

Cells: HeLa expressing GFP-LC3
Working concentrations: 1-5 µM
Incubation time: 2-7 hours
Method: Western blots and GFP-LC3 puncta

Cell Culture Assay[5]

Cells: A549 cells
Working concentration: 100 nM
Incubation time: 24 hours
Method: Western blots and electron microscopy

RELATED PRODUCTS

Product	Description	Cat. Code
HeLa-Difluo™ hLC3 Cells	Autophagy reporter cells	heldf-hlc3b
Torin 1	mTOR inhibitor	inh-tor1
RAW-Difluo™ hLC3 Cells	Autophagy reporter cells	rawdf-mlc3b

TECHNICAL SUPPORT
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