

# Rapamycin

## mTOR Inhibitor

Catalog # tlr1-rap

For research use only

Version # 10I01-MM

## PRODUCT INFORMATION

### Content:

- 5 mg Rapamycin

### Storage and stability:

- Rapamycin is provided as a solid and shipped at room temperature. Store at -20°C. Solid product is stable 1 year at -20°C.
- Upon resuspension, Rapamycin should be aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles. Resuspended product is stable for 3 months at -20°C when properly stored.

## DESCRIPTION

Rapamycin is an inhibitor of the Ser/Thr protein kinase named "mammalian target of rapamycin" (mTOR) that regulates cell growth and metabolism in response to environmental cues. mTOR is a downstream target of PI3K, an important actor in TLR signaling<sup>1</sup>. Rapamycin was shown to block TLR2- and TLR4-mediated expression of TNF- $\alpha$  and IL-6 in neutrophils stimulated with Pam3CSK4 or LPS<sup>2</sup>.

1. Kuo CC. *et al.*, 2006. J. Immunol., 176: 5943 - 5949. 2. Lorne E. *et al.*, 2009. Am. J. Respir. Cell Mol. Biol., 41: 237 - 245.

## CHEMICAL PROPERTIES

**CAS number:** 53123-88-9

**Synonym:** 23,27-Epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclohentacontine, Antibiotic AY 22989; NSC 2260804; Sirolimus

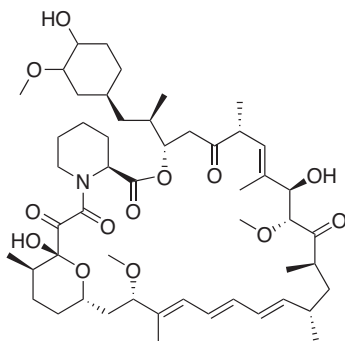
**Formula:** C<sub>51</sub>H<sub>79</sub>NO<sub>13</sub>

**Molecular weight:** 914.17

**Solubility:** 10 mM in DMSO or ethanol 2mM

**Appearance:** Off-white solid

**Purity:** >95% (HPLC)



## METHODS

### Preparation of sterile stock solution (10 mM)

To obtain a 10 mM stock solution:

1. Add 550  $\mu$ l DMSO to 5 mg rapamycin vial.
2. Vortex until complete solubilization.
3. Aliquote and store at -20°C.
4. Once rapamycin is solubilized, dilutions can be prepared by adding sterile water.

### Working concentrations:

- 10-100 nM when used as an inhibitor in the TLR signaling cascade
- 10-500 nM when used to induce autophagy

### mTOR inhibition in the TLR signaling cascade:

To assess the role of mTOR, the appropriate TLR ligand with or without rapamycin at 10- 100 nM was added to HEK-Blue™ hTLR2 or HEK-Blue™ hTLR4 cells, and then incubated at 37°C. HEK-Blue™ TLR cells are engineered HEK293 cells that stably co-express a human TLR gene and an NF- $\kappa$ B-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. To increase the sensitivity to their cognate agonists, HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells were further transfected with the co-receptors CD14 and MD2/CD14, respectively. Recognition of a TLR by its cognate receptor triggers a signaling cascade leading to the activation of NF- $\kappa$ B and the production of SEAP. SEAP levels can be determined spectrophotometrically using HEK-Blue Detection or QUANTI-Blue™, both are SEAP detection media that turn purple/blue in the presence of alkaline phosphatase.

## RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR2 Cells	hkb-htlr2
HEK-Blue™ hTLR4 Cells	hkb-htlr4
LPS-EK Ultrapure	tlrl-eklps
Pam3CSK4	tlrl-pms
QUANTI-Blue™ (5 pouches)	rep-qb1

## TECHNICAL SUPPORT

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