

## mTOR Inhibitor

Catalog # tlrl-rap

# For research use only

Version # 15C27-MM

# PRODUCT INFORMATION

#### **Contents:**

• 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 for 1 year 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:**

- The absence of bacterial contamination (e.g. endotoxins and peptidoglycans) is confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

# **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. Rapamycin is also an inducer of autophagy, as inhibition of mTOR mimics cellular starvation by blocking signals required for cell growth and proliferation¹. Furthermore, mTOR is a downstream target of PI3K, an important actor in TLR signaling². Indeed, Rapamycin can block TLR2- and TLR4-mediated expression of TNF- $\alpha$  and IL-6 in neutrophils stimulated with Pam3CSK4 or LPS³.

1. Jung CH. et al., 2010. mTOR regulation of autophagy. FEBS Lett. 584(7):1287-95.

2. Kuo CC. et al., 2006. Class I and III Phosphatidylinositol 3'-Kinase Play Distinct Roles in TLR Signaling Pathway J. Immunol., 176:5943-9.

3. Lorne E. et al., 2009. Participation of mammalian target of Rapamycin complex 1 in Toll-Like Receptor 2– and 4–induced neutrophil activation and acute lung injury. Am. J. Respir. Cell Mol. Biol., 41: 237-45.

## **CHEMICAL PROPERTIES**

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

Synonym:2327-Epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclohentriacontine,

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)

Structure:

# HO O N O O HO O O

#### **METHOD**

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

- 1. Add 550 µl DMSO to 5 mg rapamycin.
- 2. Vortex until completely solubilized.
- 3. Prepare aliquots 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-κB-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 <sup>™</sup> hTLR2 Cells HEK-Blue <sup>™</sup> hTLR4 Cells	hkb-htlr2 hkb-htlr4
LPS-EK Ultrapure Pam3CSK4	tlrl-eklps tlrl-pms
QUANTI-Blue™	rep-qb1