FK506

Calcineurin inhibitor and Autophagy inducer - InvitroFit™

Catalog code: inh-fk5-5 https://www.invivogen.com/fk506

For research use only

Version 23I14-MM

PRODUCT INFORMATION

Contents

• 5 x 10 mg FK506 - InvitroFit™

Storage and stability

- FK506 is provided as a translucent film and shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots of FK506 and store at -20°C. Avoid repeated freeze-thaw cycles. Resuspended product is stable for 1 months at -20°C when properly stored.

Quality control

- The inhibitory activity has been validated using cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue[™] TLR2 and HEK-Blue[™] TLR4 cells.

DESCRIPTION

FK506 (Tacrolimus) is commonly used as an immunosuppressant to prevent the rejection of organ transplants. Similar to cyclosporin A (CsA), FK506 blocks the activation of calcineurin through the formulation of complexes with immunophilins. FK506 binds to a different immunophilin than CsA, called FK506 binding protein (FKBP)¹. Further, it has been demonstrated that the FK506–FKBP complex suppresses the activation of the calcineurin-dependent NFAT pathway and calcineurin-independent activation pathway for JNK and p38².³.

1. Powell J.D. & Zheng Y., 2006. Dissecting the mechanism of T-cell anergy with immunophilin ligands. Curr Opin Investig Drugs. 7(11):1002-7. 2. Matsuda S. et al., 2000. Two distinct action mechanisms of immunophilinligand complexes for the blockade of T-cell activation. EMBO Rep. 1(5):428-34. 3. Vafadari R. et al., 2012. IInhibitory effect of tacrolimus on p38 mitogenactivated protein kinase signaling in kidney transplant recipients measured by whole-blood phosphospecific flow cytometry. Transplantation. 93(12):1245-51.

CHEMICAL PROPERTIES

CAS number: 104987-11-3Formula: $C_{44}H_{69}NO_{12}$ Molecular weight: 804.02 g/mol Solubility: DMSO (100 mg/ml) Working concentration: 1-100 nM

Structure:

HO, CH₃ CH₂ CH₃ CH₂ CH₃ CH

METHODS

Preparation of FK506 stock solution (20 mM)

- 1. Add 608 µl DMSO to 10 mg of FK506.
- 2. Vortex until completely resuspended.
- 3. Prepare aliquots and store stock solution at -20 °C.
- 4. Further dilutions can be prepared using aqueous buffers. We do not recommend storing the aqueous solution for more than one day.

Calcineurin signaling inhibition using Jurkat-Lucia™ NFAT cells:

Jurkat-Lucia™NFAT cells were derived from the human T Lymphocyte-based Jurkat cell line by stable integration of an NFAT-inducible Lucia reporter construct. These cells induce the activation of NFAT in response to phorbol myristate acetate (PMA) and T-lymphocyte mitogens, such as concanavalin A (ConA). Levels of Lucia luciferase are readily measurable in the cell culture supernatant when using QUANTI-Luc™ 4 Lucia/Gaussia, a luciferase detection reagent. For more information, visit www.invivogen.com/jurkat-lucia-nfat-cells.

- 1. Prepare a Jurkat-Lucia™ NFAT cell suspension ~2.5 x 10° cells/ml in
- 2. Add 20 μ l of FK506 (1-100 nM final concentration) in a well of a 96-well plate and incubate the plate for 1 h at 37 °C, 5% CO₂.

IMDM medium and add 160 µl of the cell suspension per well.

- 3. Add 20 µl of Concanavalin A (30 µg/ml final concentration) per well.
- 4. Incubate the plate for 18-24 h at 37 °C, 5% CO₂.
- 5. Determine Lucia luciferase levels using QUANTI-Luc™ 4 Lucia/ Gaussia, a coelenterazine-based luminescence assay reagent.

RELATED PRODUCTS

Product	Description	Cat. Code
Concanavalin A	NFAT activator	inh-cona
Jurkat-Lucia™ NFAT Cells	Reporter T lymphocytes	jktl-nfat
PMA	NF-ĸB Activator	tlrl-pma
QUANTI-Luc™ 4 Lucia/Gaussia	Luciferase detection reagent	rep-qlc4lg1

