Ruxolitinib
JAK1 and JAK2 Inhibitor - InvitroFit™
Catalog code: tlrl-rux
https://www.invivogen.com/ruxolitinib

For research use only
Version 2314-MM

PRODUCT INFORMATION

Contents
- 5 mg of Ruxolitinib - InvitroFit™

Storage and stability
- Ruxolitinib is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension in DMSO, prepare aliquots of ruxolitinib and store at -20°C. Avoid repeated freeze-thaw cycles. Resuspended product is stable for 6 months when properly stored.

Quality Control:
- Purity: >97% (UHPLC)
- The biological activity has been confirmed using cellular assays.
- The absence of bacterial contamination (e.g., lipopolysaccharides and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Ruxolitinib (also known as INCB018424) is a potent, reversible, and selective Janus Kinase (JAK) 1 and JAK2 inhibitor. JAKs are constitutively bound to cytokine receptors, such as Type I interferons (IFNs) and interleukin-6 (IL-6). Upon binding of the ligand to the receptor, JAKs phosphorylate downstream targets such as STAT3/5, Akt, and ERK. This induces the production of cytokines and chemokines, including IFN-stimulated genes (ISGs). JAK-STAT signaling is crucial for the regulation and homeostasis of hematopoiesis and immunity.

Ruxolitinib was developed as a potential therapeutic for a family of blood cancers termed myeloproliferative neoplasms (MPNs), which are characterized by the aberrant activation of the JAK–STAT pathway due to a mutation (V617F) in JAK2, a wild-type JAK1-2 signaling pathway. Inhibition of the JAK-STAT signaling pathway by Ruxolitinib results in a dramatic decrease in levels of inflammatory cytokines, such as IL-6 and TNF-α. It is this attenuation of the inflammatory response that the clinical efficiency of Ruxolitinib is attributed to.

Ruxolitinib competes with ATP for binding to the catalytic site in the kinase domain, and thus inhibits not only the mutated JAK2 but wild-type JAK1-2 signaling pathways. Inhibition of the JAK-STAT signaling pathway by Ruxolitinib results in a dramatic decrease in levels of inflammatory cytokines, such as IL-6 and TNF-α. It is this attenuation of the inflammatory response that the clinical efficiency of Ruxolitinib is attributed to.

Additionally, synergy has been reported between Ruxolitinib and the chemotherapy drug, dexamethasone, in the treatment of acute lymphoblastic leukemia in both in vitro and in vivo preclinical models.

CHEMICAL PROPERTIES

Structure:
- Synonym: INCB018424
- Solubility: 10 mg/ml in DMSO
- CAS number: 941678-49-5
- Formula: C17H18N6
- Molecular weight: 306.37 g/mol

METHODS

Preparation of Ruxolitinib stock solution (20 mM)
1. Add 816 µl DMSO to 5 mg ruxolitinib and vortex until completely resuspended.
2. Prepare aliquots and store at -20°C. Prepare further dilutions using aqueous buffers. Do not store aqueous dilutions for more than 1 day.

Working concentration: 100 nM-10 µM for cell culture assays

Inhibition assay
Described below is a protocol to study the JAK-STAT pathway in the interferon (IFN) regulatory factor-inducible secreted embryonic alkaline phosphatase (SEAP) reporter B16 melanocytes, B16-Blue™ ISG cells.
1. Add 20 µl of ruxolitinib (final concentration 100 nM-10 µM) per well of a flat-bottom 96-well plate.
2. Add 160 µl (~75,000 cells) of a B16-Blue™ ISG cell suspension per well.
3. Incubate for 1 hour at 37°C in a 5% CO2 incubator.
4. Add 20 µl of murine IFN-β (final concentration 100-1000 U/ml).
5. Incubate the plate at 37°C in a 5% CO2 incubator for 18-24 hours.
6. Monitor SEAP production using a SEAP detection assay, such as QUANTI-Blue™ Solution.

RELATED PRODUCTS

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<td>Amlexanox</td>
<td>TBK1/IKKa inhibitor</td>
<td>inh-amx</td>
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<tr>
<td>B16-Blue™ ISG Cells</td>
<td>Reporter melanocytes</td>
<td>bb-ikhabg</td>
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<td>QUANTI-Blue™ Solution</td>
<td>SEAP detection medium</td>
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<td>SB203580</td>
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