

# Ruxolitinib

JAK1 and JAK2 Inhibitor - InvitroFit™

Catalog code: tlrl-rux

<https://www.invivogen.com/ruxolitinib>

For research use only

Version 23114-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. lipoproteins 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<sup>1</sup>. 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<sup>2</sup>. 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<sup>1,3</sup>.

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- $\alpha$ . It is this attenuation of the inflammatory response that the clinical efficiency of Ruxolitinib is attributed<sup>2</sup>. Ruxolitinib is approved for the treatment of the MPNs, myelofibrosis and polycythemia vera<sup>3</sup>. Pre-clinical data suggest that Ruxolitinib shows potential in the treatment of inflammatory conditions such as acute graft versus host disease<sup>4</sup>. 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* pre-clinical models<sup>5</sup>.

1. Quintas-Cardama, A. *et al.*, 2010. Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms. *Blood* 115, 3109-17. 2. Ajayi S. *et al.*, 2018. Ruxolitinib. *Recent Results Cancer Res* 212, 119-132. 3. Mascarenhas J. & Hoffman R. 2012. Ruxolitinib: the first FDA approved therapy for the treatment of myelofibrosis. *Clin Cancer Res* 18:3008-14. 4. Zeiser R. *et al.*, 2020. Ruxolitinib for Glucocorticoid-Refractory Acute Graft-versus-Host Disease. *N Engl J Med* 382:1800-10. 5. Verbeke D. *et al.*, 2019. Ruxolitinib Synergizes With Dexamethasone for the Treatment of T-cell Acute Lymphoblastic Leukemia. *Hemasphere* 3:e310.

## CHEMICAL PROPERTIES

### Structure:

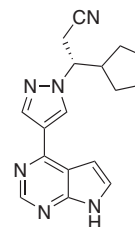
Synonym: INCB018424

Solubility: 10 mg/ml in DMSO

CAS number: 941678-49-5

Formula: C<sub>17</sub>H<sub>18</sub>N<sub>6</sub>

Molecular weight: 306.37 g/mol



## METHODS

### Preparation of Ruxolitinib stock solution (20 mM)

1. Add 816  $\mu$ l 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  $\mu$ M 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  $\mu$ l of ruxolitinib (final concentration 100 nM-10  $\mu$ M) per well of a flat-bottom 96-well plate.
2. Add 160  $\mu$ l (~75,000 cells) of a B16-Blue™ ISG cell suspension per well.
3. Incubate for 1 hour at 37°C in a 5% CO<sub>2</sub> incubator.
4. Add 20  $\mu$ l of murine IFN- $\beta$  (final concentration 100-1000 U/ml).
5. Incubate the plate at 37 °C in a 5% CO<sub>2</sub> incubator for 18-24 hours.
6. Monitor SEAP production using a SEAP detection assay, such as QUANTI-Blue™ Solution.

## RELATED PRODUCTS

Product	Description	Cat. Code
Amlxanox	TBK1/IKK $\epsilon$ inhibitor	inh-amx
B16-Blue™ ISG Cells	Reporter melanocytes	bb-ifnabg
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs
SB203580	p38 MAP kinase inhibitor	inh-sb20-5

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Asia: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)