SB203580

p38 MAP Kinase Inhibitor - InvitroFit™

Catalog code: inh-sb20-5 https://www.invivogen.com/sb203580

For research use only

Version 23I14-MM

PRODUCT INFORMATION

Contents

• 5 x 5 mg SB203580 - InvitroFit™

Storage and stability

- SB203580 is shipped at room temperature. Upon receipt, store at -20 $^{\circ}$ C. Protect from light.
- Upon resuspension, SB203580 should be stored at -20 °C. Avoid freeze-thaw cycles. Protect from light. The resuspended product is stable for 3 months at -20 °C when properly stored.

Quality control

- Purity ≥ 98% (UHPLC)
- 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

SB203580 is a pyridinyl imidazole inhibitor widely used to study the role of p38 mitogen-activated protein kinase (MAPK)¹. p38 MAPKs are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and are involved in cell differentiation, apoptosis, and autophagy.

SB203580 inhibits p38 catalytic activity by binding to the ATP binding pocket. It also inhibits the phosphorylation and activation of protein kinase B (PKB, also known as Akt)². Both kinases are involved in a wide array of signaling pathways, including the Toll-like receptor (TLR) signaling pathway³.

Moreover, several studies suggest that p38 MAPKs regulate distinct phases of autophagy. p38 can elicit autophagy via Beclin-1. Contrarily, p38 α has also been reported to inhibit autophagy by interfering with the trafficking of Atg9.

Of note, SB203580 is generally used at low concentrations (1-10 $\mu M)$, as it has been reported that at high concentrations SB203580 (>20 $\mu M)$ can induce the activation of the serine/threonine kinase Raf-1 4 .

1. Cuenda A. et al., 1995. SB203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. FEBS Lett. 364:229-33. 2. Lali FV. et al., 2000. The pyridinyl imidazole inhibitor SB203580 blocks phosphoinositidedependent protein kinase activity, protein kinase B phosphorylation, and retinoblastoma hyperphosphorylation in interleukin-2-stimulated T cells independently of p38 mitogenactivated protein kinase. J Biol Chem. 275(10):7395-402. 3. Jarnicki A.G. et al., 2008. Attenuating Regulatory T Cell Induction by TLR Agonists through Inhibition of p38 MAPK Signaling in Dendritic Cells Enhances Their Efficacy as Vaccine Adjuvants and Cancer Immunotherapeutics. J. Immunol. 180:3797-806. 4. Kalmes A et al., 1999. Raf-1 is activated by the p38 mitogen-activated protein kinase inhibitor, SB203580. FEBS Lett. 444(1):71-4.

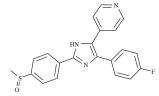
CHEMICAL PROPERTIES

Synonym: 4-(4'-Fluorophenyl)-2-(4'-methylsulfinylphenyl)-5-(4'-

pyridyl)-imidazole

CAS number: 152121-47-6 Formula: C₆H₁₆FN₃OS Molecular weight: 377.44 g/mol Solubility: 50 mg/ml in DMSO

Working concentration: 1-10 µM



Chemical structure:

MFTHODS

Preparation of stock solution (25 mM)

- 1. Add 530 µl of DMSO to 5 mg of SB203580.
- 2. Vortex until completely dissolved.
- 3. Prepare aliquots and store at -20 °C.
- 4. Once SB203580 has been resuspended, dilutions can be prepared using aqueous buffers.

p38 MAP Kinase inhibition

Inhibition of p38 MAP Kinase can be studied in a variety of cells including RAW-Lucia™ ISG cells, a reporter cell line derived from RAW 264.7 macrophages. These cells stably express an interferon regulatory factor (IRF)-inducible Lucia luciferase reporter construct. They express all TLRs (with the exception of TLR5). Stimulation of this cell line with LPS-EB activates the TLR4 pathway inducing Lucia luciferase production. The following protocol describes the monitoring of p38 MAP Kinase inhibition in RAW-Lucia™ ISG cells.

- 1. Add 20 μl of SB203580 at 1-10 μM (final concentration) per well of a flat-bottom 96-well plate.
- 2. Add 160 µl of RAW-Lucia™ ISG cell suspension (~100,000 cells) per well.
- 3. Incubate at 37°C in a 5% CO₂ incubator for 1 hour.
- 4. Add 20 μ l of LPS-EB Ultrapure at 300 ng/ml (final concentration) per well of a flat-bottom 96-well plate.
- 5. Incubate the plate at 37°C in a 5% $\rm CO_2$ incubator for 18-24 hours.
- 6. Monitor Lucia luciferase reporter protein production using a luciferase detection reagent, such as QUANTI-Luc $^{\text{TM}}$ 4 Lucia/Gaussia.

RELATED PRODUCTS

Product	Description	Cat.Code
LDC ED I III receives	TI D4i-l	Ual Oa alaa
LPS-EB Ultrapure	TLR4 agonist	tlrl-3pelps
QUANTI-Luc™ 4 Lucia/Gaussia RAW-Lucia™ ISG cells	Luciferase detection reagent Mouse macrophages	rep-qlc4lg1 rawl-isg
KAVV-LUCIA 13G CEIIS	Mouse macrophages	i avvi-isg



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