MG-132

26S proteasome inhibitor & autophagy inducer - InvitroFit™

Catalog code: tlrl-mg132-2

https://www.invivogen.com/mg-132

For research use only

Version 23L08-MM

PRODUCT INFORMATION

Contents

- 2 x 5 mg MG-132 - InvitroFit™

Storage and stability:

- MG-132 is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots of MG-132 and store at -20°C. Resuspended MG-132 is stable for 3 months when properly stored. Avoid repeated freeze-thaw cycles.

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.

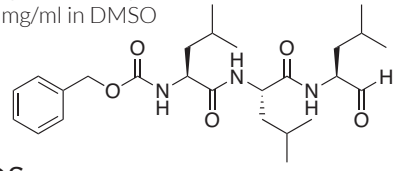
DESCRIPTION

MG-132 is a peptide aldehyde (Z-Leu-Leu-Leu-al) that selectively blocks the proteolytic activity of the 26S proteasome. This potent inhibitor is used as a tool for disrupting the proteasome-regulated degradation of intracellular proteins, such as IκB. Inhibition of IκB proteasomal degradation by MG-132 leads to the suppression of NF-κB activation. Furthermore, by blocking proteasomal degradation MG-132 can induce autophagy[2].

1. Lee DH. & Goldberg AL., 1998. Proteasome inhibitors: valuable new tools for cell biologists. Trends Cell Biol. 8(10):397-403. **2. Ge PF. et al., 2009.** Inhibition of autophagy induced by proteasome inhibition increases cell death in human SHG-44 glioma cells. Acta Pharmacol Sin. 30(7):1046-52. **3. Lee AH. et al., 2003.** Proteasome inhibitors disrupt the unfolded protein response in myeloma cells. PNAS. 100(17):9946-51. **4. Guzman ML. et al., 2001.** Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood. 98(8):2301-7.

CHEMICAL PROPERTIES

CAS number: 133407-82-6
Synonym: Z-Leu-Leu-Leu-al
Formula: $C_{26}H_{41}N_3O_5$
Molecular weight: 475.62 g/mol
Solubility: 20 mg/ml in DMSO
Structure:



METHODS

Preparation of stock solution at 20 mg/ml (42 mM)

1. Add 250 µl DMSO to 5 mg MG-132.
2. Vortex until completely dissolved.
3 Prepare aliquots of MG-132 and store at -20 °C.
4. Once MG-132 has been dissolved, dilute 1 in 100 using sterile culture medium to obtain a solution at 200 µg/ml (420 µM). Do not store dilutions for more than one day.

Note: Dilutions in water may cause the product to precipitate.

26S proteasome inhibition

Inhibition of the 26S proteasome can be studied in a variety of cells including RAW-Blue™ cells, a reporter cell line derived from RAW 264.7 macrophages. They stably express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) gene. These cells express all TLRs (with the exception of TLR5). Stimulation of this cell line with Pam3CSK4 activates the TLR2 pathway inducing SEAP production. The following protocol describes the monitoring of 26S proteasome inhibition in RAW-Blue™ cells.

1. Add 20 µl of MG-132 at 200 ng/ml - 20 µg/ml (final concentration) per well of a flat-bottom 96-well plate.
2. Add 160 µl of RAW-Blue™ cell suspension (~100,000 cells) per well.
3. Incubate at 37°C in a 5% CO_2 incubator for 1 hour.
4. Add 20 µl of Pam3CSK4 at 1 µg/ml (final concentration) per well of a flat-bottom 96-well plate.
5. Incubate the plate at 37°C in a 5% CO_2 incubator for 18-24 hours.
6. Monitor SEAP reporter protein production using a SEAP detection reagent, such as QUANTI-Blue™ Solution.

PROTOCOLS

For reference only; as described in the indicated publications.

Cell Culture Assay[2]
Cells: Human SHG-44 glioma cells
Working concentration: 6 µM
Incubation time: 48 h
Method: Cell viability assay, transmission electron microscopy

Cell Culture Assay[3]
Cells: NIH 3T3 or J558 myeloma cells
Working concentration: 0.2 - 20 µM
Incubation time: 1- 4 h
Method: Western Blot and RT-PCR analysis

Cell Culture Assay[4]
Cells: Primary human acute myelogenous leukemia (AML) cells
Working concentration: 1 µM
Incubation time: 6 h
Method: Electrophoretic mobility shift assays (EMSAs), Western Blot and RT-PCR analysis

RELATED PRODUCTS

Product	Description	Cat. Code
Pam3CSK4	TLR2 agonist	tlrl-pms
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
Rapaymcin	Autophagy inducer	tlrl-rap
RAW-Blue™ cells	Macrophage Reporter Cells	raw-sp

TECHNICAL SUPPORT
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