Research Focus

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Sonic Hedgehog:

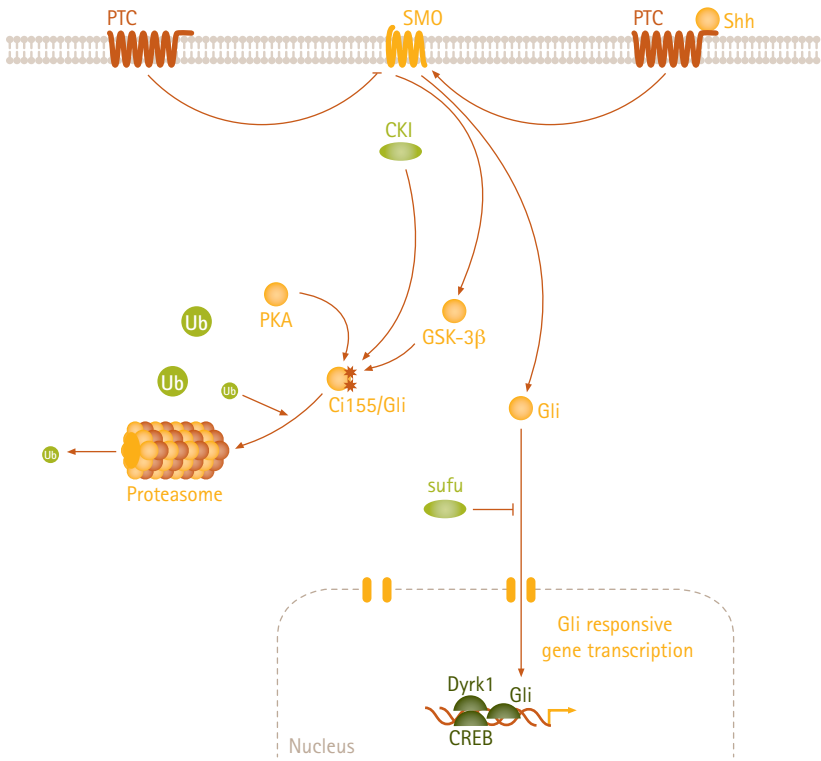
Its dual role in morphogenic and mitogenic signaling

Introduction

Mammalian Hedgehog proteins include Sonic Hedgehog (Shh), Indian Hedgehog (Ihh), and Desert Hedgehog (Dhh). They are involved in regulating pattern formation during embryonic development and in the maintenance of some tissue types in adults. Shh is expressed mainly in epithelia in teeth, hair, whiskers, gut, bladder, urethra, vas deferens, and lung. Dhh is found in Schwann and Sertoli cell precursors, and Ihh is expressed in gut and cartilage.

Molecular structure

Shh is the best-characterized Hedgehog protein. It is synthesized as a 45 kDa precursor protein, which is then autocatalytically cleaved to generate a 20 kDa N-terminal fragment that is responsible for all Hh biological activity and a 25 kDa C-terminal fragment that contains the autoprocessing unit. The N-terminal fragment of Shh contains palmitic acid and cholesterol as two lipid tethers, which allow it to remain associated with the plasma membrane. The cholesterol moiety is believed to be responsible for directing Hedgehog traffic in the secretory cells.



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Developmental roles

Shh, a secreted morphogen, has been implicated in several embryonic developmental processes. Shh signaling is required throughout embryonic development and is involved in the determination of cell fate and embryonic patterning during early vertebrate development. Disruption of Shh in humans also leads to holoprosencephaly (lack of development of forebrain in the embryo). Shh is involved in neural tube patterning and in the development of left-right symmetry. In skin, Shh is involved in maintaining the stem cell population. Shh displays inductive, proliferative, neurotrophic, and neuroprotective properties. Shh often works in concert with the Wnt signaling protein in setting embryonic patterns. During the late stage of development, Shh is involved in the proper formation of a variety of tissues and organs and it functions with other signaling molecules, such as the fibroblast growth factors and bone morphogenetic protein, to mediate developmental processes.

Proteins involved in Shh signaling

Shh often works in concert with the Wnt signaling protein in setting embryonic patterns. The Wnt pathway uses β-catenin to transduce its signals to the nucleus; however, the Shh pathway utilizes a 155 amino acid protein, Cubitus interruptus (Ci155) in *Drosophila* or Gli in mammals. Shh signaling is known to occur through a receptor complex associating two membrane proteins, Patched (Ptc) and Smoothened (Smo). Ptc is a twelve-pass membrane protein that acts as a receptor and binds Hedgehog ligand; Smo is a seven-pass membrane protein that acts as a signal transducer. In this regard, Smo displays homology to G-protein-coupled receptors that are usually associated with G-protein-coupled receptor kinases.

Signaling events in the Shh pathway

In the absence of Shh, Ptc interacts with Smo and inhibits its activity. Under these conditions, Ci is targeted for proteolysis, which generates a truncated 75-amino acid form (Ci75), which acts as a transcriptional repressor. In vertebrates, three Gli proteins (Gli1, Gli2, and Gli3) have been reported. Despite several homologous regions, including a DNA-binding domain with five C2H2 zinc fingers and a C-terminal transcription activation domain, these three proteins have distinct activities and are not considered to be functionally equivalent. Gli1 acts as a transcriptional activator and Gli3 as a repressor. Gli2 can act either as an activator or a repressor depending upon post-transcriptional and post-translational modifications. Shh binding to Ptc removes the inhibitory effect on Smo and allows Ci/Gli to enter the nucleus and act as a transcriptional activator. Smo action is mediated through a protein complex containing the kinesin-like protein Costal2 (Cos2), the Ser/Thr kinase Fused (Fu) and Ci/Gli. Transcriptional activity of Ci/Gli is also regulated through its binding to Suppressor of Fu (Sufu), which is a negative regulator of Hedgehog signaling in *Drosophila* as well as in vertebrates. It binds to all three Gli proteins with different affinities. Shh controls cell cycle progression by regulating the expression and activity of cyclins. It is also involved in expression of EGF and EGF receptor.

Phosphorylation events in Shh signaling

Protein kinase A (PKA), casein kinase I (CKI) and glycogen synthase kinase 3β (GSK-3β) play a significant role in regulating the Hedgehog signaling process. They all bind to Cos2, and phosphorylate homologous domains on Ci/Gli and Smo. Phosphorylation of Ci by PKA, CKI and GSK-3β is shown to be essential for the efficient processing of Ci155 to its transcriptional repressor form, Ci75. Inhibition of any of these kinases can lead to Ci155 accumulation. The role of phosphorylation in the regulation of vertebrate Gli proteins has not yet been clearly defined, although PKA is shown to block vertebrate Hedgehog signaling.

Roles in adult tissues

Although Hedgehog signaling is well studied during embryonic development, less is known about its role in adult tissues. Some studies have shown that Shh activity is retained by some cells in mature organs and dysregulation of activity in these cells, in some cases due to mutations in Shh pathway components, leads to tumorigenesis. Abnormal activation of the Shh pathway has been described in a variety of human cancers and in cancer stem cells. Loss of patched, over expression of Shh, and activating mutations of Gli have been reported in basal cell carcinomas, medulloblastoma, and breast carcinomas. Loss of Smo has been linked to impaired hematopoietic stem cell renewal and diminution in the induction of chronic myelogenous leukemia (CML) by Bcr-Abl. On the other hand, constitutively active Smo is shown to cause higher proliferation of CML stem cells. Amplification of Gli has also been shown in malignant gliomas and osteosarcoma. Finally, mutations in Smo and Sufu have also been associated with the formation of sporadic basal cell carcinoma and medulloblastoma. Hence, the Hedgehog pathway has become a potential target for drug development for the treatment of cancers and degenerative diseases.

SONIC HEDGEHOG ANTAGONISTS

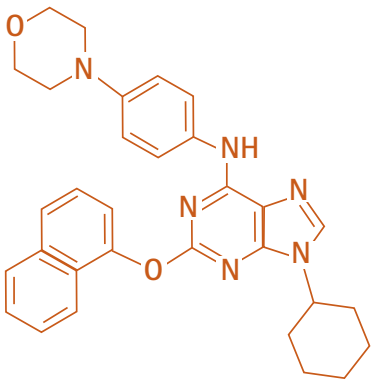
Available from: **www.millipore.com**

Product	Description	Qty/Pk	Catalogue No.
AY 9944	Specifically blocks 7-dehydro-cholesterol reductase (Δ^7-sterol reductase; DHC; IC_{50}= 13 nM) and disrupts Shh signaling during embryogenesis.	5 mg	190080
Betulinic Acid	A pentacyclic triterpene that acts as an inhibitor of Hh/Gli signaling pathway (IC_{50} = 32 µM).	5 mg	200498
Cyclopamine, *V. californicum*	A cell-permeable steroidal alkaloid and cholesterol mimic that disrupts cholesterol biosynthesis and antagonizes Shh signaling through direct interaction with Smo.	1 mg	239803
Cyclopamine-KAAD	A cell-permeable Cyclopamine analog that inhibits Hh signaling with similar or lower toxicity (IC_{50} = 20 nM in Shh-LIGHT2 assay). Binds to SmoA1 and promotes its exit from the endoplasmic reticulum.	100 mg	239804
Hh/Gli Antagonist, GANT58	A cell-permeable blocker of downstream Hh signaling that targets Gli-mediated gene transactivation (IC_{50} ~5 µM in SAG-stimulated Shh-L2 cells). Reduces cellular mRNA levels of Gli1, Hip1, & Ptch, and inhibit Gli-dependent tumor growth both *in vitro* and *in vivo*.	5 mg	373400
Hh/Gli Antagonist, GANT61	A cell-permeable compound that selectively blocks downstream Hh pathway and target Gli-mediated gene transactivation (IC_{50} ~5 µM in SAG-stimulated Shh-L2 cells).	5 mg	373401
Hh Signaling Antagonist VII, JK184	A cell-permeable downstream Hh pathway blocker that directly targets the enzymatic activity of Adh7 (IC_{50} = 210 nM). Inhibits Hg-Ag-induced Gli-transcription activity (IC_{50} = 30 nM) as well as Gli1 and Ptc1 mRNA expression in a dose-dependent manner in H3H10T1/2 cells.	5 mg	373385
Hedgehog Antagonist VIII	A cell-permeable compound that potently inhibits OCT-Shh-stimulated Gli transcription activity in a 10t1/2(s12) cell-based Luciferase reporter assay (IC_{50} = 70 nM).	5 mg	373402
Hh Signaling Antagonist X, Itraconazole	A cell-permeable inhibitor of Hh signaling (IC_{50} ~0.8 µM against SHHN-induced Gli transcription activity in Shh-Light2 cells) that acts in a Ptch-independent manner (IC_{50} ~0.9 µM against Ptch promoter-mediated transcription activity in murine Ptch-/- fibroblasts). Presumably acts by binding smoothened (smo) at a site distinct from that targeted by cyclopamine and SAG.	250 mg	419825
Hh Signaling Antagonist XII, HPI-1	A cell-permeable downstream inhibitor of Hh signaling that effectively inhibits Gli-dependent transcription activity in both *Sufu*$^{+/+}$ NIH 3T3 (shh-LIGHT2) and Sufu-/- fibroblast cultures (IC_{50} = 1.5 & 3 µM, respectively).	10 mg	373275
Hh Signaling Antagonist XIII, HPI-3	A cell-permeable blocker of downstream Hh signaling. Inhibits Gli-dependent transcription activity in both *Sufu*$^{+/+}$ NIH 3T3 (shh-LIGHT2) and *Sufu*$^{-/-}$ fibroblast cultures (IC_{50} = 3 & 9 µM, respectively).	10 mg	373276
Hh Signaling Antagonist XIV, SANT-2	A highly potent Smo antagonist (Kd = 12 nM) that displays allosteric binding characteristics similar to SANT-1.	10 mg	373273
Hh Signaling Antagonist XV	A potent blocker of Hh-Ag1.5 to Smo (IC_{50} = 5 nM in CHO-K1 membrane expressing murine Smo). Blocks Gli-mediated transcription activity upon Hh-Ag1.5 stimulation in TM3-based reporter assays (IC_{50} = 2.7 or 35 nM against 1 or 25 nM Hh-Ag1.5, respectively).	10 mg	374274
Jervine	A cell-permeable steroidal alkaloid similar to cyclopamine that induces cyclopia by blocking Shh signaling (IC_{50} ~500-700 nM in s12 cells).	1 mg	420210
SANT-1	A potent antagonist of Shh signaling (IC_{50} = 20 nM in Shh-LIGHT2 assay and in Ptch1$^{-/-}$ cells cells) that acts by binding to Smo (K_d = 1.2 nM). Inhibits the activities of both wild type and oncogenic Smo with equal potency (IC_{50} = 30 nM in SmoA1-LIGHT2 assay).	5 mg	559303
Tomatidine, HCl	A steroidal alkaloid that structurally resembles cyclopamine, but lacks the capacity to inhibit Sonic Hedgehog (Shh) signaling. Useful as a negative control.	25 mg	614350
U18666A	A cell-permeable amphiphilic amino-steroid that alters intracelluar membrane protein trafficking by impairing intracellular biosynthesis and transport of LDL-derived cholesterol. A weak inhibitor of Shh signaling	10 mg	662015
Veratramine, HCl, *V. californicum*	A cell-permeable steroidal alkaloid that is structurally related to and serves as a suitable inactive control for cyclopamine, cyclopamine-KAAD, and jervine in Shh signaling.	5 mg	676925

Purmorphamine, 5 mg

(Qty: 5 mg, Catalogue No. 540220)

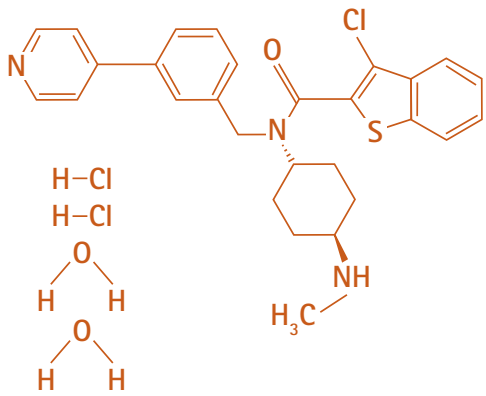
A cell-permeable purine compound that promotes the differentiation of multipotent mesenchymal progenitor cells (EC_{50} = 1 µM) into osteoblasts. Directly binds to and activates Smo. Purity: ≥98% by HPLC.



Smoothened Agonist, SAG

(Qty: 1 mg, Catalogue No. 566660)

A cell-permeable benzothiophene compound that modulates the coupling of Smo with its downstream effector by interacting with the Smo heptahelical domain (Kd = 59 nM). Induces Smo internalization and activates Hh signaling (EC_{50} ~3 nM in NIH 3T3-derived Shh-LIGHT2 cells) and counteracts Cyclopamine-KAAD inhibition of Smo. Purity: ≥98% by HPLC.

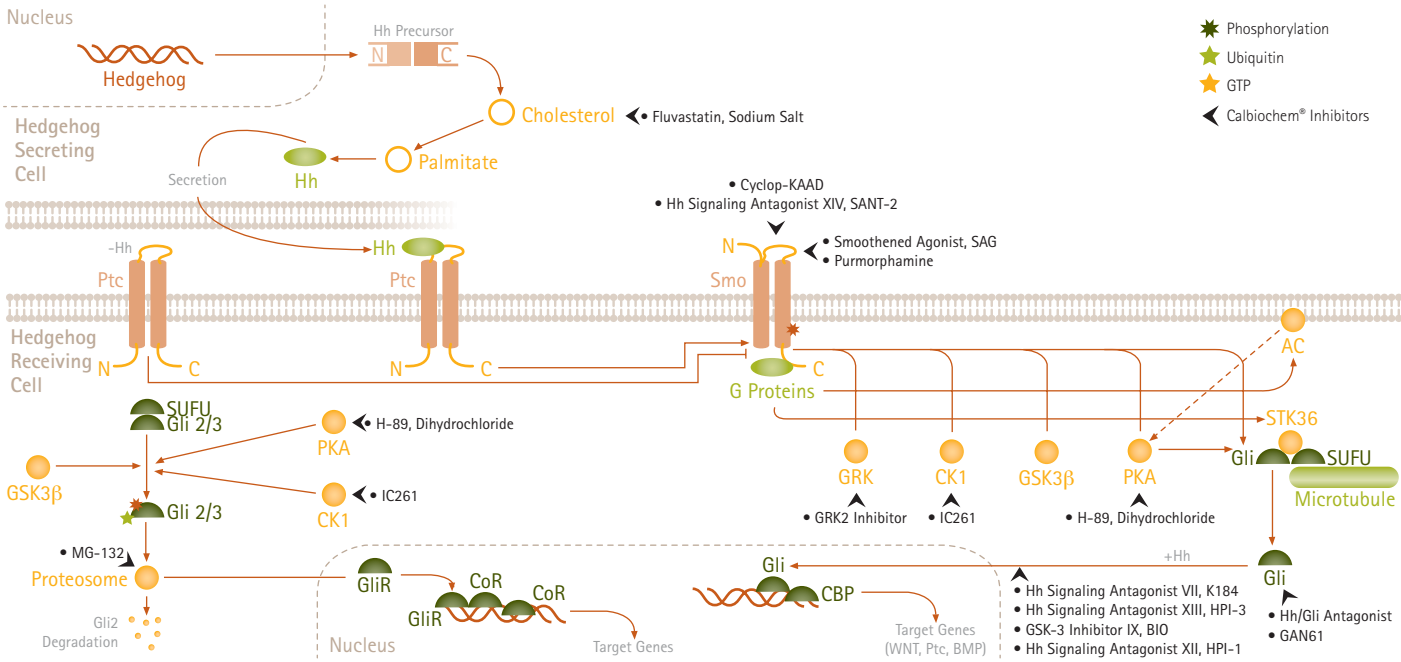


Hh Signaling Pathway Modulators Panel

(Catalogue No. 373386)

A panel consisting of 14 potent, selective, and cell-permeable antagonists, inhibitors and agonists that are useful for the study of the Hedgehog signaling pathway. Because Hh signaling involves both intercellular and intracellular signaling, this panel provides a convenient way to simultaneously interrogate signal-secreting and signal-receiving cells in one experiment. The panel contains the following inhibitors and 15 mL anhydrous DMSO for reconstitution:

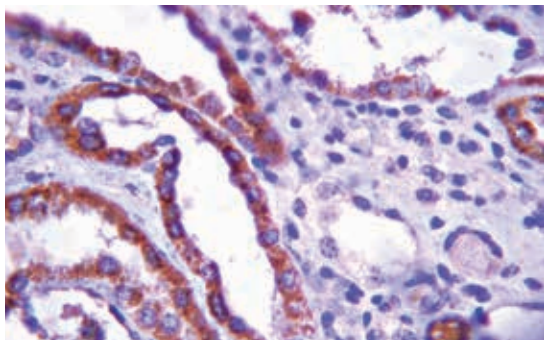
Components	Target	Amount	Catalogue No.
Smoothened Agonist, SAG	Smo agonist; Hh pathway activator	1 mg	566660
Cyclopamine-KAAD	Smo antagonist	100 µg	239804
Hh Signaling Antagonist XIV, SANT-2	Smo antagonist, targets different site than Cyclopamine	10 mg	373273
Hh/Gli Antagonist, GANT61	Inhibitor of Gli binding to DNA	5 mg	373401
GSK-3 Inhibitor IX, BIO	Reduces Gli1-dependent transcriptional activity	1 mg	361550
H-89, Dihydrochloride	PKA Inhibitor; Hh agonist	1 mg	371963
IC261	CK1 Inhibitor (CK1δ and CK1ε; IC_{50} = 0.7-1.3 µM and = 0.6-1.4 µM, respectively)	5 mg	400090
GRK2 Inhibitor	Reduces phosphorylation of human Smo	5 mg	182200
MG-132	Proteasomal Inhibitor	5 mg	474790
Hh Signaling Antagonist VII, JK184	Targets Adh7, inhibits Gli-transcription activity, and downregulates the expressions of Gli1 and Ptc1	5 mg	373385
Purmorphamine	Smo agonist, targets Cylopamine binding site and upregulates Gli1 and Ptc1	5 mg	540220
Fluvastatin, Sodium Salt	Cholesterol biosynthesis inhibitor	25 mg	344095
Hh Signaling Antagonist XII, HPI-1	Remains effective against $Sufu^{-/-}$ fibroblasts overexpressing Gli1 or Gli2	10 mg	373275
Hh Signaling Antagonist XIII, HPI-3	Ineffective against $Sufu^{-/-}$ fibroblasts over-expressing Gli1 or Gli2	10 mg	373276
DMSO	-	15 mL	KP31817



Anti-Protein patched homolog 1

(Qty: 100 µg, Catalogue No. 06-1102)

Hedgehog signaling is regulated by its receptor, Protein patched homolog 1, which keeps the pathway turned off in the absences of activation. The immunogen used to develop this purified rabbit polyclonal antibody was a KLH-conjugated linear peptide corresponding to the cytoplasmic domain of human Protein patched homolog 1. Recognizes the cytoplasmic domain of patched homolog 1 in human and mouse. **Applications**: Western blot, IHC

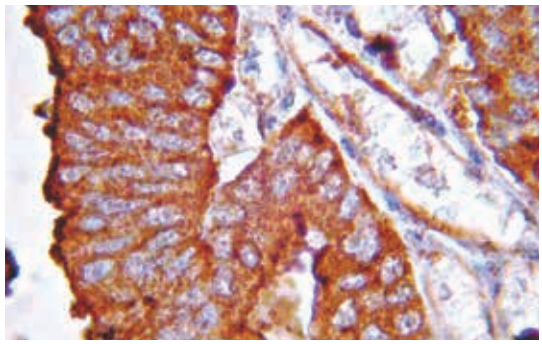


Immunohistochemistry of paraffin-embedded kidney tissue using Anti-Protein patched homolog 1 shows that expression is restricted to proximal tubules with no distal tubule involvement. Immunostaining was performed using a 1:300 dilution of Catalogue No. 06-1102, Anti-Protein patched homolog 1. Reactivity was detected using the IHC-Select Detection Kit (Catalogue No. DAB050).

Anti-Sonic Hedgehog, affinity-purified rabbit polyclonal

(Qty: 100 µL, Catalogue No. 06-1106)

Immunogen used was GST-tagged recombinant protein corresponding to the N-terminus of Sonic Hedgehog. Recognizes the N-terminus of Sonic Hedgehog in human and mouse. **Applications**: Western blot, IHC



Immunohistochemistry using a 1:500 dilution of Catalogue No. 06-1106, Anti-Sonic Hedgehog was performed on a section of paraffin-embedded colorectal carcinoma tissue. Reactivity was detected using the IHC Select® Detection Kit (Catalogue No. DAB050).

Anti-Smo Mouse mAb (1D9)

(Qty: 100 µg, Catalogue No. ST1718)

Immunogen used was a full-length, recombinant, human Smo (aa 653-787) expressed as a GST fusion protein. Recognizes 75 kDa Smo in A431 cells.

Applications: Western blot

Anti-Sonic Hedgehog (C-product), clone EP1190Y, rabbit monoclonal

(Qty: 100 µL, Catalogue No. 04-971)

Immunogen used was a synthetic peptide corresponding to residues within the c-product subunit of human Shh protein. Recognizes both full-length (50 kDa) and c-product subunit (27 kDa) of human Shh protein.

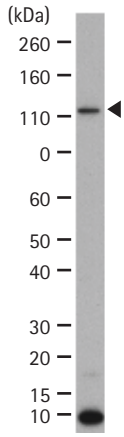
Applications: Flow cytometry, Western blot, IHC (paraffin), IP.

Anti-Protein patched homolog 2

(Qty: 100 µg, Catalogue No. 06-1103)

Protein patched homolog 2 and Smoothened form a membrane complex that serves as a receptor for Shh. The immunogen used to develop this purified rabbit polyclonal antibody was a KLH-conjugated linear peptide corresponding to human Protein patched homolog 2 at and around the extracellular domain. Recognizes Protein patched homolog 2 in human, canine, equine, and mouse.

Applications: Western blot, IHC



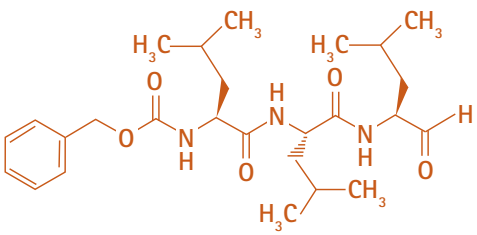
HeLa cell lysate probed with Anti-Protein patched homolog 2 (0.5 µg/mL). Proteins were visualized using a Donkey Anti-Rabbit IgG conjugated to horseradish peroxidase and a chemiluminescence detection system. Arrow indicates Protein patched homolog 2 (~130 kDa).

MG-132 (Z-LLL-CHO)

(Qty: 1 mg, 5 mg, 10 mg, 20 mg; Catalogue No. 474790)

A potent, reversible, and cell-permeable proteasome inhibitor (Ki = 4 nM), MG-132 reduces the degradation of ubiquitin-conjugated proteins in mammalian cells and permeable strains of yeast by the 26S complex without affecting its ATPase or isopeptidase activities.

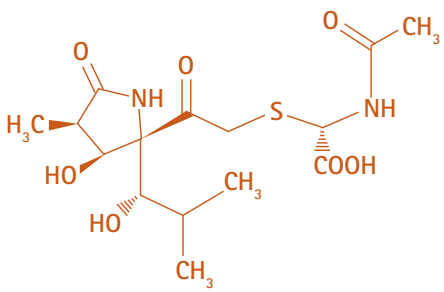
Purity: ≥98% by HPLC.



Lactacystin, Synthetic

(Qty: 200 µg, 400 µg, 1 mg; Catalogue No. 426100)

An irreversible proteasome inhibitor, this Streptomyces metabolite is a highly specific inhibitor of the 20S proteasome (MCP; multicatalytic proteinase complex) (IC_{50} = 500 nM for 20S proteasome). It blocks proteasome activity by targeting the catalytic β-subunit and is a covalent inhibitor of the chymotrypsin and trypsin-like activities of the proteasome.



References

1. Merchant, A.A., and Matsui, W. Clin. Cancer Res. 2010; 16: 3130.
2. Zhao, C., et al. Nature 2009; 458: 776.
3. Aikin, R.A., et al. EMBO Reports 2008;9:330.
4. Riobo, N.A., and Manning, D.R. Biochem. J. 2007; 403:369
5. Athar, M., et al. Exp. Dermatol. 2006; 15:667.
6. Jiang, J. Cell Cycle 2006; 5:2457.
7. Simms-Mourtada, J., et al. Clin. Cancer Res. 2006; 12:6565
8. Riobo, N.A., et al. Proc. Natl. Acad. Sci. USA 2006; 103:4505.
9. Macaron, N.C., et al. Arch. Dermatol. 2005; 141:259.
10. Benson, R.A., et al. Mol. Immunol. 2004; 41: 715.
11. Magliano, M.P., and Hebrok, M. Nat. Rev. Cancer 2003; 3:903.
12. Ruizi Altaba A., et al. Nat. Rev. Cancer 2002; 2:361.
13. Taylor, M.D., et al. Nat. Genet. 2002; 31:306.
14. Reinferberger, J., et al. Cancer Res. 1998; 58:1798.
15. Dahmane, N., et al. Nature 1997;389: 876.
16. Oro. A., et al. Science 1997; 276: 817.

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