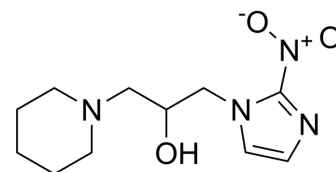


Pimonidazole hydrochloride

Cat. No.:	HY-105129
CAS No.:	70132-51-3
Molecular Formula:	C ₁₁ H ₁₉ ClN ₄ O ₃
Molecular Weight:	290.75
Target:	Others
Pathway:	Others
Storage:	4°C, sealed storage, away from moisture * In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture)



H-Cl

SOLVENT & SOLUBILITY

In Vitro	DMSO : 250 mg/mL (859.85 mM; Need ultrasonic)					
	H ₂ O : 5 mg/mL (17.20 mM; ultrasonic and warming and heat to 80°C)					
	Preparing Stock Solutions	Solvent	Mass	1 mg	5 mg	10 mg
		Concentration				
		1 mM		3.4394 mL	17.1969 mL	34.3938 mL
5 mM			0.6879 mL	3.4394 mL	6.8788 mL	
	10 mM		0.3439 mL	1.7197 mL	3.4394 mL	
Please refer to the solubility information to select the appropriate solvent.						
In Vivo	1. Add each solvent one by one: 50% PEG300 >> 50% saline Solubility: 10 mg/mL (34.39 mM); Clear solution; Need ultrasonic					

BIOLOGICAL ACTIVITY

Description	Pimonidazole is a novel hypoxia marker for complementary study of tumor hypoxia and cell proliferation in tumor ^[1] . Pimonidazole accumulates in hypoxic cells via covalent binding with macromolecules or by forming reductive metabolites after reduction of its nitro group, it can be used for qualitative and quantitative assessment of tumor hypoxia ^[2] .
In Vitro	Pimonidazole, the exogenous hypoxia marker, is a 2-nitroimidazole compound, which forms covalent bonds with cellular macromolecules at oxygen levels below 1.3%. Detection: Hypoxic cells were recognized by immunohistochemical detection of pimonidazole using a mouse monoclonal antibody. Cell proliferation was detected with a commercially available monoclonal antibody for proliferating cell nuclear antigen (PCNA). Assessment of hypoxia and cell proliferation was made qualitatively with light microscopy and quantitatively using point counting and image analysis software methods. The pimonidazole-protein adducts were then detected by immunofluorescence Cells (90% confluent) are treated with pimonidazole hydrochloride (10-100 μM), diluted in complete media or PBS, and placed in hypoxia or normoxia for 2-4 hours.

Then, cells are washed 4 times in HBSS, fixed for 10 min with 10% neutral buffered formalin at room temperature, washed 3 times with tris buffered saline, and mounted with Prolong Gold with DAPI mounting media^[3]. To monitor the intrinsic hypoxic signal, the tissue is removed and the embryos incubated with 400 μ M pimonidazole for 2 h before fixation. Immunostaining is performed using antibodies against pimonidazole-protein adducts (Hypoxyprobe)^[4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo

Pimonidazole is a 2-nitroimidazole that is reductively activated specifically in hypoxic cells and forms stable adducts with thiol groups in proteins, peptides, and amino acids. Additionally, the amount of pimonidazole that is detected is directly proportional to the level of hypoxia within tumors in vivo.

The following protocol is provided for this useful tool to interrogate the levels of hypoxia in vivo.

1. Pimonidazole hydrochloride is suspended at a concentration of 30 mg/ml in 0.9% sterile saline or PBS.
 2. Multiple mice are injected intravenously (tail vein) with 60 mg/kg of the pimonidazole solution.
 3. Let circulate in vivo for 90 min before the mice are euthanized with isoflurane or carbon dioxide (CO₂). Note: For animal euthanasia: In compliance with proper IACUC protocols!
 4. Mice are sacrificed and organs are then removed, weighed, snap-frozen, and stored in plastic scintillation vials at -80 °C.
 5. The pimonidazole stain can work on paraffin-embedded tissue, frozen tissue, and cell lines. Organs are embedded in OCT, placed in a cryostat, and cut into 10 μ m-thick sections. Sections are placed on microscope slides for staining.
- MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Theranostics. 2022 Apr 4;12(7):3196-3216.
- Asian J Pharm Sci. 2023 Mar 20.
- Sci Rep. 2023 Apr 21;13(1):6528.

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- [1]. Varia MA, et al. Pimonidazole: a novel hypoxia marker for complementary study of tumor hypoxia and cell proliferation in cervical carcinoma. *Gynecol Oncol*. 1998 Nov;71(2):270-7.
- [2]. Masaki Y, et al. Imaging Mass Spectrometry Revealed the Accumulation Characteristics of the 2-Nitroimidazole-Based Agent "Pimonidazole" in Hypoxia. *PLoS One*. 2016 Aug 31;11(8):e0161639.
- [3]. Kristina Y Aguilera, et al. Hypoxia Studies with Pimonidazole in vivo. *Bio Protoc*
- [4]. Stephanie M Evans, et al. Molecular probes for imaging of hypoxia in the retina. *Bioconjug Chem*. . 2014 Nov 19;25(11):2030-7.
- [5]. Stephanie M Evans, et al. Molecular probes for imaging of hypoxia in the retina. *Bioconjug Chem*. . 2014 Nov 19;25(11):2030-7.

Caution: Product has not been fully validated for medical applications. For research use only.

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