产品名称：MLN4924
产品别名：Pevonedistat

生物活性：

Description	Pevonedistat (MLN4924) is a potent and selective NEDD8-activating enzyme (NAE) inhibitor with an IC_{50} of 4.7 nM.
IC_{50} & Target	IC50: 4.7 nM (NAE)[1]
In Vitro	Pevonedistat (MLN4924) is a potent inhibitor of NAE, and is selective relative to the closely related enzymes UAE, SAE, UBA6 and ATG7 (IC_{50}=1.5, 8.2, 1.8 and >10 μM, respectively) when evaluated in purified enzyme assays that monitor the formation of E2-UBL thioester reaction products. Pevonedistat (MLN4924) selectively inhibits NAE activity compared to the closely related ubiquitin-activating enzyme (UAE, also known as UBA1) and SUMO-activating enzyme (SAE; a heterodimer of SAE1 and UBA2 subunits), in purified enzyme and cellular assays. MLN4924 exhibits potent cytotoxic activity against a variety of human tumour-derived cell lines[1]
In Vivo	Pevonedistat (MLN4924) (sc, 10 mg/kg, 30 mg/kg, or 60 mg/kg) inhibits the NEDD8 pathway resulting in DNA damage in Mice bearing HCT-116 xenografts[1] Pevonedistat (sc, 120 mg/kg) and TNF-α (10 μg/kg) synergistically cause liver damage in SD rats[2]

Solvent&Solubility

In Vitro:

DMSO： ≥ 50 mg/mL (112.73 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.2547 mL	11.2734 mL	22.5469 mL
	5 mM	0.4509 mL	2.2547 mL	4.5094 mL
	10 mM	0.2255 mL	1.1273 mL	2.2547 mL

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 **In Vitro** 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (5.64 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.64 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline)

Solubility: ≥ 2.5 mg/mL (5.64 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.64 mM，饱和度未知) 的澄清溶液。

	以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (5.64 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.64 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Soucy TA, et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature. 2009 Apr 9;458(7239):732-6. [2]. F S Wolenski, et al. The NAE inhibitor pevonedistat (MLN4924) synergizes with TNF-α to activate apoptosis. Cell Death Discovery 1, Article number: 15034 (2015)

实验参考：

Cell Assay	HCT-116 cells grown in 6-well cell-culture dishes are treated with 0.1% DMSO (control) or 0.3 μM Pevonedistat (MLN4924) for 24 h. Whole cell extracts are prepared and analysed by immunoblotting. For analysis of the E2-UBL thioester levels, lysates are fractionated by non-reducing SDS-PAGE and immunoblotted with polyclonal antibodies to Ubc12, Ubc9 and Ubc10. For analysis of other proteins, lysates are fractionated by reducing SDS-PAGE and probed with primary antibodies as follows: mouse monoclonal antibodies to CDT1, p27, geminin, ubiquitin, securin/PTTG and p53 or rabbit polyclonal antibodies to NRF2, Cyclin B1 and GADD34[1]
Animal Administration	Mice[1] Mice bearing HCT-116 tumours of 300-500 mm^3 are administered a single Pevonedistat (MLN4924) dose (of 10, 30 or 60 mg/kg), and tumors are excised at various time-points over the subsequent 24 h period. The relative levels of NEDD8-cullin and NRF2 are estimated by quantitative immunoblot analysis using Alexa680-labelled anti-IgG as the secondary antibody. The statistical difference between the groups for NEDD8-cullin inhibition is determined using the Kruskal-Wallis test. For the analysis of CDT1 and phosphorylated CHK1 (Ser317) levels in tumour sections, formalin-fixed, paraffin-embedded tumour sections are stained with the relevant antibodies, amplified with HRP-labelled secondary antibodies and detected with the ChromoMap DAB Kit. Slides are counterstained with haematoxylin. Images are captured using an Eclipse E800 microscope and Retiga EXi colour digital camera and processed using Metamorph software. CDT1 and phosphorylated CHK1 levels are expressed as a function of the DAB signal area. Rats[2] Ten-week-old male Sprague-Dawley rats are used. Across two studies, a total of eight animals in each group are dosed with vehicle, TNF-α, Pevonedistat (MLN4924), or Pevonedistat (MLN4924)+TNF-α. Animals are first intravenously administered either vehicle (1×PBS) or 10 μg/kg TNF-α. One hour later, they are subcutaneously administered vehicle (20% sulfobutyl ether beta-cyclodextrin in 50 mM citrate buffer, pH 3.3) or 120 mg/kg Pevonedistat (MLN4924). Scheduled euthanasia occurred 24 h postdose. Unscheduled euthanasia is performed when animals exhibited moribund conditions. Serum is collected at necropsy and analyzed by Idexx Laboratories for serum chemistry markers of liver damage. Additionally, the livers from five animals in each group are removed, separated into two sections and either frozen at -80°C for subsequent protein analysis or fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4-6 μm, mounted on glass

	slides, stained with hematoxylin and eosin, and analyzed with an Olympus BX51 light microscope for histopathology assessment. Microscopic findings are recorded in concordance with the standardized nomenclature for classifying lesions within the livers of rats.
References	[1]. Soucy TA, et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature. 2009 Apr 9;458(7239):732-6. [2]. F S Wolenski, et al. The NAE inhibitor pevonedistat (MLN4924) synergizes with TNF-α to activate apoptosis. Cell Death Discovery 1, Article number: 15034 (2015)