产品名称：KU-55933 (ATM Kinase Inhibitor)
产品别名：KU-55933

生物活性：

Description	KU-55933 is a potent ATM inhibitor with an IC_{50} and K_i of 12.9 and 2.2 nM, respectively, and is highly selective for ATM as compared to DNA-PK, PI3K/PI4K, ATR and mTOR.			
IC_{50} & Target [1]	ATM	DNA-PK	mTOR	PI3K
	12.9 nM (IC_{50})	2500 nM (IC_{50})	9300 nM (IC_{50})	16600 nM (IC_{50})
In Vitro	KU-55933 (10 μM) blocks the ionizing radiation-induced p53 serine 15 phosphorylation. KU-55933 has a dose-dependent effect in inhibiting this ATM-dependent phosphorylation event with an estimated IC50 of 300 nM. KU-55933 ablates the ionizing radiation-induced phosphorylation of these ATM substrates. KU-55933 specifically inhibits ATM but not the other DNA damage-activated PIKKs, ATR, and DNA-PK[1]. KU-55933 induces pATM, p53, E2F1 and pATR, noticeably upregulates the nuclear fraction of E2F1 at the 0.5 h time point[2]. Metformin increases ATM and AMPK phosphorylation, as well as SHP protein level in primary hepatocytes, and this stimulatory effect of metformin is repressed by a specific ATM kinase inhibitor KU-55933[3].			

Solvent&Solubility

In Vitro:

DMSO： 80 mg/mL (202.28 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.5285 mL	12.6425 mL	25.2851 mL
	5 mM	0.5057 mL	2.5285 mL	5.0570 mL
	10 mM	0.2529 mL	1.2643 mL	2.5285 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 (6.32 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (6.32 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 (6.32 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (6.32 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 (6.32 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.32 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]. Hickson I, et al. Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. Cancer Res. 2004 Dec 15;64(24):9152-9 [2]. Khalil HS, et al. Pharmacological inhibition of ATM by KU55933 stimulates ATM transcription.Exp Biol Med (Maywood). 2012 Jun;237(6):622-34. Epub 2012 Jun 22. [3]. Kim YD, et al. Orphan nuclear receptor SHP negatively regulates growth hormone-mediated induction of hepatic gluconeogenesis through inhibition of STAT5 transactivation.J Biol Chem. 2012 Sep 12.

实验参考：

Cell Assay	1BR or AT4 cells are seeded in 10-cm Petri dishes and treated on day 2 (80 to 90% confluence). Cells are preincubated for 1 hour with KU-55933 or vehicle control and then exposed to 5 Gy of ionizing radiation. Time courses of cell cycle distribution are performed, and the optimal time for discrimination of populations is selected as 16 hours. All subsequent experiments are performed at the 16-hour time point. Cells are stained with propidium iodide according to standard protocols and analyzed by FACS with a FACScalibur. Exponentially growing (50-70% confluent) SW620 cells in 60 mm dishes are exposed to KU-55933 or DMSO for 1 h before addition of etoposide (final concentration of 0.1 and 1 μM) for 16 h before harvesting, propidium iodide staining and analysis as above. [1]
Kinase Assay	ATM for use in the in vitro assay is obtained by immunoprecipitation with rabbit polyclonal antiserum raised to the COOH-terminal 400 amino acids of ATM in buffer containing 25 mM HEPES (pH 7.4), 2 mM $MgCl_2$, 250 mM KCl, 500 μM EDTA, 100 μM Na_3VO_4, 10% v/v glycerol, and 0.1% v/v Igepal. ATM-antibody complexes are isolated from nuclear extract by incubating with protein A-Sepharose beads for 1 hour and then through centrifugation to recover the beads. In the well of a 96-well plate, ATM-containing Sepharose beads are incubated with 1 μg of substrate glutathione S-transferase-p53N66 (NH_2-terminal 66 amino acids of p53 fused to glutathione S-transferase) in ATM assay buffer [25 mM HEPES (pH 7.4), 75 mM NaCl, 3 mM $MgCl_2$, 2 mM $MnCl_2$, 50 μM Na_3VO_4, 500 μM DTT, and 5% v/v glycerol] at 37°C in the presence or absence of inhibitor. After 10 minutes with gentle shaking, ATP is added to a final concentration of 50 μM and the reaction continued at 37°C for an additional 1 hour. The plate is centrifuged at 250×g for 10 minutes (4°C) to remove the ATM-containing beads, and the supernatant is removed and transferred to a white opaque 96-well plate and incubated at room temperature for 1.5 hours to allow glutathione S-transferase-p53N66 binding. This plate is then washed with PBS, blotted dry, and analyzed by a standard ELISA technique with a phospho-serine 15 p53 antibody. The detection of phosphorylated glutathione S-transferase-p53N66 substrate is performed in combination with a goat antimouse horseradish peroxidase-conjugated secondary antibody. Enhanced chemiluminescence solutionis used to produce a signal and chemiluminescent detection is carried out via a TopCount plate reader. [1]
	[1]. Hickson I, et al. Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. Cancer Res. 2004 Dec 15;64(24):9152-9

References	[2]. Khalil HS, et al. Pharmacological inhibition of ATM by KU55933 stimulates ATM transcription.Exp Biol Med (Maywood). 2012 Jun;237(6):622-34. Epub 2012 Jun 22. [3]. Kim YD, et al. Orphan nuclear receptor SHP negatively regulates growth hormone-mediated induction of hepatic gluconeogenesis through inhibition of STAT5 transactivation.J Biol Chem. 2012 Sep 12.