产品名称：**RepSox**
产品别名：**RepSox**

生物活性：

Description	RepSox is a potent and selective of the TGFβR-1/ALK5 inhibitor which inhibits ALK5 autophosphorylation with IC_{50} of 4 nM.
IC_{50} & Target	IC50: 4 nM (ALK5 autophosphorylation)[1]
In Vitro	RepSox also inhibits ATP binding to ALK5 with IC_{50} of 23 nM. RepSox shows potent activity in both binding and cellular assays and exhibits selectivity over p38 mitogen-activated protein kinase. with IC_{50} of >16 μM[1]. RepSox act s as an inhibitor of the Tgfβ1 kinase. Treatment with 25 μM RepSox almost completely eliminates Smad3 phosphorylation, indicating that RepSox strongly inhibits Tgfβ signaling in somatic cells. RepSox is most effective at replacing Sox2 during days 10-11 after transduction and that therefore cultures of Oct4, Klf4, and cMyc-transduced MEFs give rise to intermediates capable of responding to RepSox treatment. These intermediates appear at day 4 post-transduction and peak at days 10-11. Treatment with RepSox decreased the proportion of cells in G2/M phase of the cell cycle, indicating it does not increase the proliferation rate of these partially reprogrammed cells[2].
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 52 mg/mL (180.98 mM)** * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	3.4804 mL	17.4022 mL	34.8044 mL
	5 mM	0.6961 mL	3.4804 mL	6.9609 mL
	10 mM	0.3480 mL	1.7402 mL	3.4804 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: ≥ 7.5 mg/mL (26.10 mM); Clear solution

此方案可获得 ≥ 7.5 mg/mL (26.10 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 75.0 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% corn oil

Solubility: ≥ 7.5 mg/mL (26.10 mM); Clear solution

此方案可获得 ≥ 7.5 mg/mL (26.10 mM，饱和度未知) 的澄清溶液。

	以 1 mL 工作液为例，取 100 μL 75.0 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。
References	[1]. Gellibert F, et al. Identification of 1,5-naphthyridine derivatives as a novel series of potent and selective TGF-beta type I receptor inhibitors. J Med Chem. 2004 Aug 26;47(18):4494-506. [2]. Ichida JK, et al. A small-molecule inhibitor of tgf-Beta signaling replaces sox2 in reprogramming by inducing nanog. Cell Stem Cell. 2009 Nov 6;5(5):491-503.

实验参考：

Cell Assay	To test anti-TGF-β activity of compounds, HepG2 cells are seeded in 96 well microplates at a concentration of 35000 cells per well in 200 μL of serum-containing medium. The microplates are then placed for 24 h in a cell incubator at 37°C, 5% CO_2 atm. RepSox dissolved in DMSO are then added at concentrations of 50 nM to 10 μM (final concentration of DMSO 1%) for 30 min prior to the addition of recombinant TGF-β (1 ng/mL). After an overnight incubation, the cells are washed with PBS and lysed by addition of 10 μL of passive lysis buffer. Inhibition of luciferase activity relative to control groups is used as a measure of compound activity. A concentration−response curve is constructed from which an IC_{50} value is determined graphically [1].
Kinase Assay	The kinase domain of ALK5 is cloned by PCR and expressed in a baculovirus/Sf9 cells system. The protein is 6-His tagged in the C terminus and purified by affinity chromatography using a Ni^{2+}column, and the obtained material is used to assess compound activity in an autophosphorylation assay. Purified enzyme (10 nM) is incubated in 50 μL of Tris buffer (Tris 50 mM, pH 7.4; NaCl, 100 mM; $MgCl_2$, 5 mM; $MnCl_2$, 5 mM; and DTT, 10 mM). The enzyme is preincubated with different concentrations of RepSox (0.1% DMSO final concentration in the test) for 10 min at 37°C. The reaction is then initiated by the addition of 3 μM ATP (0.5 μCi γ-33P-ATP). After 15 min at 37°C, phosphorylation is stopped by the addition of SDS−PAGE sample buffer (50 mM Tris-HCl, pH 6.9, 2.5% glycerol, 1% SDS, and 5% β-mercaptoethanol). The samples are boiled for 5 min at 95°C and run on a 12% SDS−PAGE. Dried gels are exposed to a phosphor screen overnight. ALK5 autophosphorylation is quantified using a Storm imaging system [1].
References	[1]. Gellibert F, et al. Identification of 1,5-naphthyridine derivatives as a novel series of potent and selective TGF-beta type I receptor inhibitors. J Med Chem. 2004 Aug 26;47(18):4494-506. [2]. Ichida JK, et al. A small-molecule inhibitor of tgf-Beta signaling replaces sox2 in reprogramming by inducing nanog. Cell Stem Cell. 2009 Nov 6;5(5):491-503.