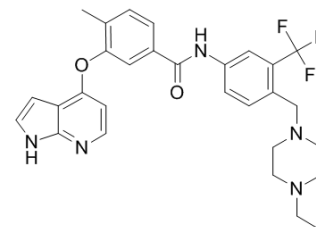


## NG25

<b>Cat. No.:</b>	HY-15434		
<b>CAS No.:</b>	1315355-93-1		
<b>Molecular Formula:</b>	C <sub>29</sub> H <sub>30</sub> F <sub>3</sub> N <sub>5</sub> O <sub>2</sub>		
<b>Molecular Weight:</b>	537.58		
<b>Target:</b>	MAP4K; MAP3K		
<b>Pathway:</b>	MAPK/ERK Pathway		
<b>Storage:</b>	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month



## SOLVENT & SOLUBILITY

### In Vitro

DMSO : ≥ 100 mg/mL (186.02 mM)  
 \* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	1.8602 mL	9.3009 mL	18.6019 mL
	5 mM	0.3720 mL	1.8602 mL	3.7204 mL
	10 mM	0.1860 mL	0.9301 mL	1.8602 mL

Please refer to the solubility information to select the appropriate solvent.

### In Vivo

- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
 Solubility: ≥ 2.5 mg/mL (4.65 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)  
 Solubility: 2.5 mg/mL (4.65 mM); Suspended solution; Need ultrasonic
- Add each solvent one by one: 10% DMSO >> 90% corn oil  
 Solubility: ≥ 2.5 mg/mL (4.65 mM); Clear solution

## BIOLOGICAL ACTIVITY

### Description

NG25 is a potent dual TAK1 and MAP4K2 inhibitor, with IC<sub>50</sub>s of 149 nM and 21.7 nM, respectively.

### IC<sub>50</sub> & Target

MAP4K2 21.7 nM (IC <sub>50</sub> )	TAK1 149 nM (IC <sub>50</sub> )	LYN 12.9 nM (IC <sub>50</sub> )	GSK 56.4 nM (IC <sub>50</sub> )
ABL,ARG 75.2 nM (IC <sub>50</sub> )	FER 82.3 nM (IC <sub>50</sub> )	SRC 113 nM (IC <sub>50</sub> )	Eph B2 672 nM (IC <sub>50</sub> )

	ZAK 698 nM (IC <sub>50</sub> )	Eph A2 773 nM (IC <sub>50</sub> )	Eph B4 999 nM (IC <sub>50</sub> )	ZC1/HGK 3250 nM (IC <sub>50</sub> )
	RAF1 7590 nM (IC <sub>50</sub> )			
<b>In Vitro</b>	<p>NG25 is a potent dual TAK1 and MAP4K2 inhibitor, with IC<sub>50</sub>s of 149 nM and 21.7 nM, respectively. NG25 also potently suppresses several kinases such as LYN, CSK, FER, p38<math>\alpha</math>, ABL, ARG and SRC, with IC<sub>50</sub>s of 12.9, 56.4, 82.3, 102, 75.2, and 113 nM, respectively<sup>[1]</sup>. NG25 is very potent suppressor of CpG B- or CpG A-stimulated secretion of IFN<math>\alpha</math> and CL097-stimulated secretion of IFN<math>\beta</math>, with complete inhibition by 400 nM<sup>[2]</sup>. NG25 treatment reduces cell viability of all tested breast cancer cell lines in a dose dependent manner. NG25 (2 <math>\mu</math>M) enhances the cytotoxic effect of Dox on breast cancer cells<sup>[3]</sup>.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>			

## PROTOCOL

### Kinase Assay <sup>[2]</sup>

IRF7 is expressed in Escherichia coli as a glutathione S-transferase (GST) fusion protein with a PreScission proteinase cleavage site between the GST and the IRF7. The GST-IRF7 is captured on glutathione-Sepharose and IRF7 released from GST and glutathione-Sepharose by digestion with PreScission proteinase. His6-tagged IKK $\beta$  and TBK1 are expressed in their active phosphorylated forms in insect Sf21 cells and purified by affinity chromatography on nickel nitrilotriacetate-agarose. Active GST-IKK $\alpha$  is purchased from Millipore and assayed.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

### Cell Assay <sup>[2]</sup>

3.5 $\times$ 10<sup>5</sup> Gen2.2 cells or Flt3-DCs are incubated for 1 h in 96-well plates without or with the indicated concentrations of inhibitor, then stimulated with 1  $\mu$ M CpG (type A or B) or 1  $\mu$ g/mL of CL097 or R848. After 5 or 12 h the cell culture supernatants are collected, clarified by centrifugation, and frozen at -80°C until cytokine levels are analyzed. For cell viability assays, unstimulated cells are incubated for 12 h in the absence or presence of inhibitors. Cells are then fixed and the percentage of live cells analyzed by flow cytometry.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## CUSTOMER VALIDATION

- Nat Cell Biol. 2017 Oct;19(10):1248-1259.
- Cell Death Differ. 2019 Dec;26(12):2520-2534.
- J Cell Biol. 2018 Aug 6;217(8):2727-2742.
- Oncogene. 2017 Oct 5;36(40):5620-5630.
- Sci Signal. 2018 Jul 10;11(538). pii: eaan5850.

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## REFERENCES

- [1]. Tan L, et al. Discovery of type II inhibitors of TGF $\beta$ -activated kinase 1 (TAK1) and mitogen-activated protein kinase kinase kinase kinase 2 (MAP4K2). J Med Chem. 2015 Jan 8;58(1):183-96.
- [2]. Pauls E, et al. Essential role for IKK $\beta$  in production of type 1 interferons by plasmacytoid dendritic cells. J Biol Chem. 2012 Jun 1;287(23):19216-28.
- [3]. Wang Z, et al. TAK1 inhibitor NG25 enhances doxorubicin-mediated apoptosis in breast cancer cells. Sci Rep. 2016 Sep 7;6:32737.

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

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