# NG25

Cat. No.:	HY-15434		
CAS No.:	1315355-93-1		
Molecular Formula:	$C_{29}H_{30}F_{3}N_{5}O_{2}$		
Molecular Weight:	537.58		
Target:	МАР4К; МАРЗК		
Pathway:	MAPK/ERK Pathway		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year

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# SOLVENT & SOLUBILITY

In Vitro	DMSO : 16.67 mg/mL (31.01 mM; ultrasonic and warming and heat to 60°C)				
	Preparing Stock Solutions	Solvent Mass Concentration	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	1. 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				
	2. 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				
	<ol> <li>Add each solvent one by one: 10% DMSO &gt;&gt; 90% corn oil Solubility: ≥ 2.5 mg/mL (4.65 mM); Clear solution</li> </ol>				

BIOLOGICAL ACTIV	ITV			
DIOLOGICALACITY				
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> )

# Product Data Sheet

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	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> )			
In Vitro	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, p38α, 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 IFNα and CL097-stimulated secretion of IFNβ, 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 µM) enhances the cytotoxic effect of Dox on breast cancer cells <sup>[3]</sup> . MCE has not independently confirmed the accuracy of these methods. They are for reference only.			

#### 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 IKKβ and TBK1 are expressed in their active phosphorylated forms in insect Sf21 cells and purified by affinity chromatography on nickel nitrilotriacetate-agarose. Active GST-IKKα 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>	3.5×10 <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 μM CpG (type A or B) or 1 μg/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.
- Proc Natl Acad Sci U S A. 2023 Dec 12;120(50):e2313148120.
- Cell Death Dis. 2022 Apr 30;13(4):421.
- Oncogene. 2017 Oct 5;36(40):5620-5630.

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#### REFERENCES

[1]. Tan L, et al. Discovery of type II inhibitors of TGFβ-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 IKKβ 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.

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

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