**Proteins** 

# **Screening Libraries**

# JC-1

Cat. No.: HY-15534 CAS No.: 3520-43-2 Molecular Formula:  $C_{25}H_{27}Cl_{4}IN_{4}$ Molecular Weight: 652.23

Target: Fluorescent Dye

Pathway: Others

Storage: 4°C, sealed storage, away from moisture and light

\* In solvent: -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture

and light)

**Product** Data Sheet

# **SOLVENT & SOLUBILITY**

In Vitro

DMSO: 5 mg/mL (7.67 mM; ultrasonic and warming and heat to 60°C)

H<sub>2</sub>O: < 0.1 mg/mL (insoluble)

Preparing Stock Solutions	Solvent Mass Concentration	1 mg	5 mg	10 mg
	1 mM	1.5332 mL	7.6660 mL	15.3320 mL
	5 mM	0.3066 mL	1.5332 mL	3.0664 mL
	10 mM			

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: 1.25 mg/mL (1.92 mM); Suspended solution; Need ultrasonic
- 2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: 1.25 mg/mL (1.92 mM); Suspended solution; Need ultrasonic

### **BIOLOGICAL ACTIVITY**

Description

JC-1 (CBIC2) is an ideal fluorescent probe widely used to detect mitochondrial membrane potential. JC-1 accumulates in mitochondria in a potential dependent manner and can be used to detect the membrane potential of cells, tissues or purified mitochondria. In normal mitochondria, JC-1 aggregates in the mitochondrial matrix to form a polymer, which emits strong red fluorescence (Ex=585nm, Em=590nm); When the mitochondrial membrane potential is low, JC-1 cannot aggregate in the matrix of mitochondria and produce green fluorescence (ex=514nm, em=529nm)<sup>[1]</sup>.

In Vitro

JC-1 staining

a. Take the 6-well plate as an example for cell planking, and the density is  $5\times10^5/\text{mL}$ . Incubate overnight in 5% CO<sub>2</sub> incubator

Note: it is suggested that the cell density during apoptosis induction should not exceed  $1\times10^6/\text{ml}$ , which can also be cultured

to the appropriate density according to your own cell type.

- b. Take 0.5 mL suspension into sterile centrifuge tube; 400 g centrifugation for 3-5 min; Discard the supernatant.
- c. The cells were resuspended with 1mljc-1 working solution and incubated in 5% CO<sub>2</sub> incubator at 37⊠ for 15-30 min.
- d. Centrifugation at room temperature for 5 min at 400 g; Suck of the supernatant.
- e. The cells were resuspended with 2 mL cell culture medium or buffer, and then centrifuged at room temperature for 5 min at 400 g; Discard the supernatant and repeat twice.
- $f. \ Resuspend \ the \ cells \ with \ 1mL \ of \ fresh \ culture \ medium \ or \ buffer, \ and \ immediately \ conduct \ subsequent \ flow \ cytometry \ or \ fluorescence \ microscope \ observation.$
- g. Data analysis (flow cytometry): mitochondria of healthy cells containing red JC-1 aggregates were detected by FL2 channel; Apoptotic or unhealthy cells containing green JC-1 monomer were detected by FL1 (FITC) channel.
- h. If used for enzyme labeling instrument, use 300  $\mu$ L buffer resuspended cells; Then 100 per hole  $\mu$  Transfer the stained cells to a light tight 96 well plate with the amount of L, and then conduct fluorescent enzyme label plate analysis.

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

# **CUSTOMER VALIDATION**

- Mol Cancer. 2019 Apr 10;18(1):85.
- Bioact Mater. 2022 Aug 11;21:20-31.
- ACS Nano. 2023 Aug 30.
- ACS Nano. 2023 Jul 11.
- ACS Nano. 2022 Mar 28.

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### **REFERENCES**

[1]. A Perelman, et al. JC-1: alternative excitation wavelengths facilitate mitochondrial membrane potential cytometry. Cell Death Dis. 2012 Nov 22;3:e430.

[2]. Vera C. Keil, et al. Ratiometric high-resolution imaging of JC-1 fluorescence reveals the subcellular heterogeneity of astrocytic mitochondria. Pflügers Archiv - European Journal of Physiology. 2011,462(5): 693-708.

[3]. Jung-Ho LEE, et al. Real-time analysis of amyloid fibril formation of  $\alpha$ -synuclein using a fibrillation-state-specific fluorescent probe of JC-1. Biochem. J. 2009, 418:311-323.

[4]. Salvioli S, et al. JC-1, but not DiOC6(3) or rhodamine 123, is a reliable fluorescent probe to assess delta psi changes in intact cells: implications for studies on mitochondrial functionality during apoptosis. FEBS Lett. 1997 Jul 7;411(1):77-82.

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

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