Data Sheet (Cat.No.T20549)

TargetMol

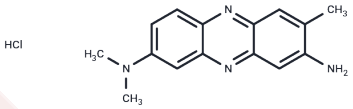
Neutral red

Chemical Properties

CAS No. : 553-24-2

Formula: C15H17ClN4

Molecular Weight: 288.77



Storage: Keep away from direct sunlight
Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	Neutral red is a vital dye, is used as an indicator and biological stain.
Targets(IC50)	Others
Cell Research	Cell Neutral red uptake assay Procedure: 1.Cells were cultured in a 96-well plate under normal conditions until they reached a confluency of 70-80%. 2.The neutral red working solution (prepared the day before the neutral red uptake assay and incubated overnight in a 37°C cell culture incubator) was centrifuged at 4,000 g for 5 minutes at room temperature (20-30°C) to remove any precipitate. 3.200 μL PBS was added to each well of the 96-well plate to rinse the cells. 4.100 μL of neutral red working solution was added to each well using a dispenser. 5.Incubated in a 37°C incubator (5% CO2) for 2-4 hours. Note: For RD and MRC-5 cell lines, the optimal incubation time is 2 hours, and longer incubation times will result in significant sensitivity loss. For Vero E6 and A549-hACE2 cell lines, the incubation time can be up to 4 hours without affecting the detection sensitivity; 6. Add 200 μL PBS to each well to wash the cells and remove the neutral red working solution. 7. Aspirate the wash buffer and gently pour the remaining buffer on a paper towel or let it air dry. Note: The wells need to be completely dry before adding neutral red destaining solution. 8. Add 100 μL of neutral red destaining solution (usually 50% ethanol, 1% acetic acid or DMSO) to each well. Shake the plate quickly at 200 rpm on a microplate shaker for 15 minutes to allow the destaining solution to completely extract neutral red from the cells and form a uniform solution. 9. Measure the absorbance of the neutral red extract at 540 nm using a spectrophotometer. The above information is based on published literature. Experimental procedures should be appropriately modified to meet specific research demands.

Solubility Information

Solubility	DMSO: 49 mg/mL (169.69 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween-80+45% Saline: 2 mg/mL (6.93 mM),Sonication is recommended. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	3.463 mL	17.3148 mL	34.6296 mL
5 mM	0.6926 mL	3.463 mL	6.9259 mL
10 mM	0.3463 mL	1.7315 mL	3.463 mL
50 mM	0.0693 mL	0.3463 mL	0.6926 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Rodrigues RM, et al. Neutral Red Uptake Assay to Assess Cytotoxicity In Vitro. Methods Mol Biol. 2023;2644:237-245.

Ellen Borenfreund, et al. A simple quantitative procedure using monolayer cultures for cytotoxicity assays (HTD/NR-90). Journal of tissue culture methods volume 9, pages7-9 (1985).