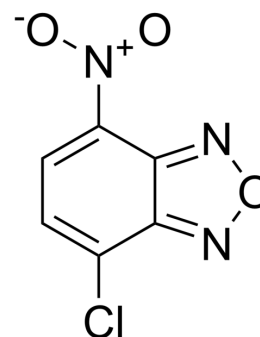


NBD-Cl

Cat. No.:	HY-D0042
CAS No.:	10199-89-0
Molecular Formula:	C ₆ H ₂ ClN ₃ O ₃
Molecular Weight:	200
Target:	Fluorescent Dye
Pathway:	Others
Storage:	4°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



SOLVENT & SOLUBILITY

In Vitro	DMSO : ≥ 100 mg/mL (500.00 mM) H ₂ O : < 0.1 mg/mL (insoluble) * "≥" means soluble, but saturation unknown.																							
	<table border="1"> <thead> <tr> <th rowspan="2">Preparing Stock Solutions</th> <th>Solvent Concentration</th> <th>Mass</th> <th>1 mg</th> <th>5 mg</th> <th>10 mg</th> </tr> </thead> <tbody> <tr> <td>1 mM</td> <td>5.0000 mL</td> <td>25.0000 mL</td> <td>50.0000 mL</td> <td></td> <td></td> </tr> <tr> <td>5 mM</td> <td>1.0000 mL</td> <td>5.0000 mL</td> <td>10.0000 mL</td> <td></td> <td></td> </tr> <tr> <td>10 mM</td> <td>0.5000 mL</td> <td>2.5000 mL</td> <td>5.0000 mL</td> <td></td> <td></td> </tr> </tbody> </table> <p>Please refer to the solubility information to select the appropriate solvent.</p>	Preparing Stock Solutions	Solvent Concentration	Mass	1 mg	5 mg	10 mg	1 mM	5.0000 mL	25.0000 mL	50.0000 mL			5 mM	1.0000 mL	5.0000 mL	10.0000 mL			10 mM	0.5000 mL	2.5000 mL	5.0000 mL	
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In Vivo	1. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (12.50 mM); Clear solution																							

BIOLOGICAL ACTIVITY

Description	NBD-Cl is a nonfluorescent reagent which becomes highly fluorescent after reaction with thiol or amino groups ^[1] .
In Vitro	NBD-Cl (NBD chloride) forms highly fluorescent derivatives for detection of all the protein amino acids. In addition, NBD-Cl provides a simple and sensitive method for determination of N-terminal amino acids. The differences in intensity and color of fluorescence can be used to advantage to identify prolyl peptides ^[1] . MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay ^[1]	Fluorescence Measurements ^[1]
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Fluorescence spectra are recorded on a Hitachi MPF-24 Fluorescence Spectrophotometer. The emission spectrum is determined at the optimal wavelength of 464 nm. Thin-layer chromatography is carried out on Brinkmann glass supported analytical silica G thin-layer plates without fluorescent indicator. The NBD derivatives are visualized on thin-layer plates either by examination under an ultraviolet lamp with a long wavelength filter or by using the same lamp with a 2×2-in. Corning 5-57 filter and viewing the spots through a 2×2-in. Corning 3-68 filter. The former method produces a more intense fluorescence, but the latter produces a much greater contrast appearing as white spots against a dark background. NBD-Cl (NBD chloride) is allowed to react with amino acids and peptides under the conditions. Into 3 mL Pyrex test tubes are pipette the following: (1) 2.0 mL of ethanol, (2) 0.1 mL NBD-Cl solution in ethanol 1.41 µg/mL, (3) 0.1 mL ethanol saturated with sodium acetate, (4) 0.1 mL of amino acid solution 400-600µg/mL, and the mixture incubated at 75°C for 20 min. For reaction with peptides, 1.5 mL of ethanol, 0.1 mL ethanolic sodium acetate, 0.4 mL ethanolic NBD-Cl, 1.41 µg/mL, are added to 0.4 mL of the peptide and incubated under the same conditions.

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

CUSTOMER VALIDATION

- Biosens Bioelectron. 2021 Apr 19;184:113235.

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REFERENCES

[1]. Fager RS, et al. The use of NBD chloride (7 chloro-4-nitrobenzo-2-oxa-1,3-diazole) in detecting amino acids and as an N-terminal reagent. Anal Biochem. 1973 May;53(1):290-4.

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

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