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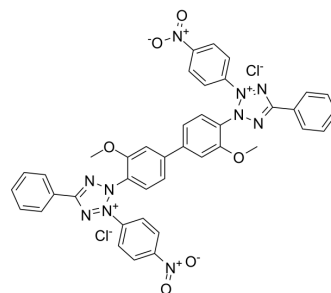
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Nitro blue tetrazolium chloride

Cat. No.:	HY-15925
CAS No.:	298-83-9
Molecular Formula:	C ₄₀ H ₃₀ Cl ₂ N ₁₀ O ₆
Molecular Weight:	818
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)



SOLVENT & SOLUBILITY

In Vitro

70%DMF : 100 mg/mL (122.25 mM; Need ultrasonic)
H₂O : 8 mg/mL (9.78 mM; Need ultrasonic)
DMSO : 5 mg/mL (6.11 mM; ultrasonic and warming and heat to 60°C)

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	1.2225 mL	6.1125 mL	12.2249 mL
	5 mM	0.2445 mL	1.2225 mL	2.4450 mL
	10 mM	0.1222 mL	0.6112 mL	1.2225 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 (3.06 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
Solubility: ≥ 2.5 mg/mL (3.06 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% corn oil
Solubility: ≥ 2.5 mg/mL (3.06 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Nitro blue tetrazolium chloride (NBT) is a substrate for dehydrogenases; is used with the alkaline phosphatase substrate 5-Bromo-4-Chloro-3-Indolyl Phosphate (BCIP) in western blotting and immunohistological staining procedures^[1].

In Vitro

Protocol for Staining with NBT and BCIP
1. Materials
Dimethylformamide (DMF, HY-Y0345).

Nitro blue tetrazolium chloride (NBT, HY-15925) Stock Solution: Dissolve 0.5 g of NBT in 10 mL of 70% DMF in water.

BCIP Stock Solution: Dissolve 0.5 g of BCIP in 10 mL 100% DMF.

Substrate Buffer: 100 mM diethanolamine buffer (pH 9.5) containing 100 mM NaCl and 5 mM MgCl₂.

2.Method

2.1 To prepare the NBT/BCIP solution, add 66 µL of stock NBT to 10 mL of substrate Buffer and mix well. Add 33 µL of stock BCIP to the solution. Use the NBT/BCIP solution within 1 hour.

2.2 Place blot in a suitable container and add the NBT/BCIP Solution. Use 10 mL of solution for a 15 × 15cm² membrane. Incubate blot at room temperature with agitation until the bands are suitably dark. Typically, ~30 minutes is required for development.

2.3 Remove the NBT/BCIP Solution and rinse blot with water to stop the reaction.

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

CUSTOMER VALIDATION

- Nat Commun. 2021 Jun 21;12(1):3803.
- Cell Death Dis. 2021 May 8;12(5):462.
- J Plant Physiol. 25 August 2023, 154076

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REFERENCES

[1]. Trinh le A, et al. Fluorescent in situ hybridization employing the conventional NBT/BCIP chromogenic stain. Biotechniques. 2007 Jun;42(6):756-9.

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

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