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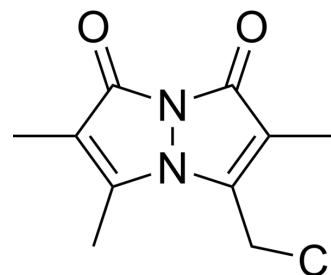
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Monochlorobimane

Cat. No.:	HY-101899
CAS No.:	76421-73-3
Molecular Formula:	C ₁₀ H ₁₁ ClN ₂ O ₂
Molecular Weight:	227
Target:	Fluorescent Dye
Pathway:	Others
Storage:	-20°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



SOLVENT & SOLUBILITY

In Vitro	DMSO : 50 mg/mL (220.26 mM; Need ultrasonic)					
	Preparing Stock Solutions	Solvent Concentration	Mass	1 mg	5 mg	10 mg
		1 mM		4.4053 mL	22.0264 mL	44.0529 mL
		5 mM		0.8811 mL	4.4053 mL	8.8106 mL
		10 mM		0.4405 mL	2.2026 mL	4.4053 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 (11.01 mM); Clear solution; Need ultrasonic					
	2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: 2.5 mg/mL (11.01 mM); Suspended solution; Need ultrasonic					

BIOLOGICAL ACTIVITY

Description	Monochlorobimane (Chlorobimane) is a fluorescent dye (λ_{ex} =380 nm, λ_{em} =470 nm) to measure glutathione (GSH) in cellular assays ^[1] .
In Vitro	Guidelines (Following is our recommended protocol. This protocol only provides a guideline, and should be modified according to your specific needs). 1. Preparation of control and experimental wells: The experiment should consist of parallel negative, positive and experimental wells respectively. Experimental wells: add 200 μ L of cell culture media containing your GSH effector of interest at desired concentration (e.g. 200 μ M H ₂ O ₂) 2. Incubate the plate overnight at 37°C, 5% CO ₂ . The incubation time varies depended on your normal protocol 3. Monochlorobimane (mBCl) is added to the cells at a final concentration of 20-100 μ M from a working solution of 1 mM. The

stock solution of mBCL (50 mM) was prepared in dimethyl sulphoxide (DMSO) and stored at -20°C; the working solution was prepared before use by diluting the stock solution in 0.1 M PBS buffer (pH 7.0). In the assay the final concentration of DMSO was below 0.2% (v/v)

4. The cell suspensions were incubated with mBCL in the dark at 25°C for ~2 h

5. Remove the dye and treatment by centrifugation at 700 X g for 5 min. Add 200 µL of the Assay Buffer to each well and continue to the preferred method of detection

6. Detection of intracellular GSH:

Fluorescence plate reader: For adherent cells; read fluorescence directly off the culturing plate. If working with suspension cells; aliquot 200 µL of the culture suspension into the white opaque plate and record fluorescence at $E_{\lambda}/E_M = 380/465 \pm 20$ nm respectively (blue).

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

PROTOCOL

Kinase Assay ^[1]

Monochlorobimane (mCB) is added to the second half liver tissue homogenate to a final concentration of 100 mM along with glutathione S-transferase (1 U/mL) obtained from equine liver; the homogenate is then allowed to incubate at room temperature for 30 min. The Glutathione (GSH)-Monochlorobimane adduct is measured in a microtiter reader^[1].

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

CUSTOMER VALIDATION

- Int J Biol Sci. 2022 Jul 11;18(11):4545-4559.
- Int J Mol Sci. 2023 Jul 28, 24(15), 12104.
- Food Chem Toxicol. 2023 Apr 18;113785.

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REFERENCES

[1]. Kamencic H, et al. Monochlorobimane fluorometric method to measure tissue glutathione. Anal Biochem. 2000 Nov 1;286(1):35-7.

[2]. Manuela D. Machado, et al. Assessment of cellular reduced glutathione content in Pseudokirchneriella subcapitata using monochlorobimane. Journal of Applied Phycology volume 24, pages1509–1516(2012).

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

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