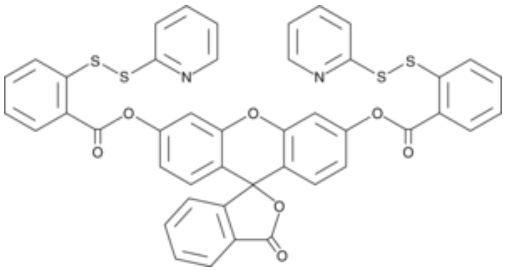
Product Data Sheet

Product Name: WSP-5
Cat. No.: GC45162

Chemical Properties

Cas. No.	1593024-78-2		
Chemical Name	3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-3',6'-diyl bis(2-(pyridin-2-yldisulfanyl)benzoate)		
SMILES	O=C(OC1=CC=C2C(OC3=C(C24C5=CC=CC=C5C(O4)=O)C=CC(OC(C6=CC=CC=C6SSC7=CC=CC=N7)=O)=C3)=C1)C8=C(SSC9=NC=CC=C9)C=CC=C8		
Formula	$C_{44}H_{26}N_2O_7S_4$	M.Wt	822.9
Solubility	25 mg/ml in DMSO, 30 mg/ml in DMF	Storage	Store at -20°C; protect from light
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure			

Protocol

This plan only provides a guide, please modify it to meet your specific needs.

1.Solution Preparation:

(1) Stock Solution Preparation: Equilibrate solid WSP-5 to room temperature and dissolve it in DMSO to prepare a stock solution with a concentration of 10 mM. Note: Unused stock solution should be aliquoted and stored in the dark at -20°C or -80°C to avoid repeated freeze-thaw cycles.

(2) Working Solution Preparation: Dilute the stock solution with an appropriate buffer (e.g., serum-free

culture medium or PBS) to the desired working concentration, such as 10-100 μM. Note: Optimal working concentrations should be adjusted based on experimental conditions or consulted literature for gradient concentration settings. Working solutions must be freshly prepared.

2.Detection of Cellular H_2S Using WSP-5 [1] (Reference Only)

(1) Cell Treatment: Culture 2.5×10^5 4T1 cancer cells in a 24-well plate and allow them to adhere for 12 hours.

(2) WSP-5 Incubation: Incubate the cells with 100 μM WPS-5 probe for 30 minutes.

(3) Na_2S Treatment:Treat the cells with 200 μM Na_2S.

(4) Fluorescence Detection:After 10 minutes, acquire fluorescence images using confocal laser scanning microscopy (CLSM). λ ex/em = 502/525 nm.

3.Analysis of Obtained Fluorescence Intensity Data to Evaluate WSP-5 Sensitivity to H_2S [2] (Reference Only)

(1) Experimental Preparation: Use black, clear-bottom 96-well plates for the experiment. Prepare a final volume of 200 μL in each well.

Prepare PBS containing 1 mM CTAB as the buffer for all experiments.

(2) Probe Solubilization: Dissolve the WSP-5 probe in PBS containing 1 mM CTAB to a final concentration of 10 μM.

(3) H_2S Donor Addition: Add increasing concentrations of Na_2S or DAT as the H_2S donor to the wells containing the WSP-5 probe. Concentrations range from 0 to 10,000 μM.

(4) Fluorescence Measurement: Measure the fluorescence intensity at regular intervals (e.g., every 30 seconds) using a SpectraMax M5 microplate reader. λex/em = 502/525 nm.

(5) Data Collection: Record the fluorescence intensity measurements over a period of 30 minutes.

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Precautions:This experimental protocol provides guidance for detecting cellular H_2S using WSP-5 and subsequent fluorescence measurements. Adjustments can be made according to specific experimental requirements.

References:

[1] He T , Qin X , Jiang C ,et al.Tumor pH-responsive metastable-phase manganese sulfide nanotheranostics for traceable hydrogen sulfide gas therapy primed chemodynamic therapy[J].Theranostics, 2020, 10(6):2453-2462.

[2] Yingzhu Zhou, Dr. Federico Mazur,et al. Sensitivity and Selectivity Analysis of Fluorescent Probes for Hydrogen Sulfide Detection[J]. Chemistry – An Asian Journal
March 1, 2022.

Background

WSP-5 (Washington State Probe-5) is a fluorescent probe containing active disulfide groups, specifically designed for rapid detection of hydrogen sulfide (H_2S) in biological samples and within cells. H_2S, like nitric oxide, has significant effects on mammalian immune, nervous, cardiovascular, and pulmonary systems. Through a reaction-based fluorescence turn-on strategy, WSP-5 selectively and rapidly reacts with H_2S, generating fluorescence with excitation and emission maxima at 502 and 525 nm, respectively[1]. Compared with the three H_2S probes WSP-1, CAY and P3, WSP-5 has the widest linear range of sensitivity, minimal interference to thiols and other analytes, the most stable in different pH environments, and can be used in Imaging H_2S in live cell systems [2].

References:

[1] Peng, B., Chen, W., Liu, C., et al. Fluorescent probes based on nucleophilic substitution-cyclization for hydrogen sulfide detection and bioimaging[J]. Chemistry 20(4), 1010-1016 (2014).

[2] Yingzhu Zhou, Dr. Federico Mazur,et al. Sensitivity and Selectivity Analysis of Fluorescent Probes for Hydrogen Sulfide Detection[J]. Chemistry – An Asian Journal
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