

Product Data Sheet

Product Name: WSP-1
Cat. No.: GC45161

Chemical Properties

Cas. No. 1352750-34-5

Chemical Name 3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl 2-(pyridin-2-yl)disulfanylnzoate

SMILES O=C(OC1=CC=C2C(OC3=C(C24C5=CC=CC=C5C(O4)=O)C=CC=C3)=C1)CO=C(OC1=CC=C2C(OC3=C(C24C5=CC=CC=C5C(O4)=O)C=CC(OC)=C3)=C1)C6=C(SSC7=NC=CC=C7)C=CC=C6

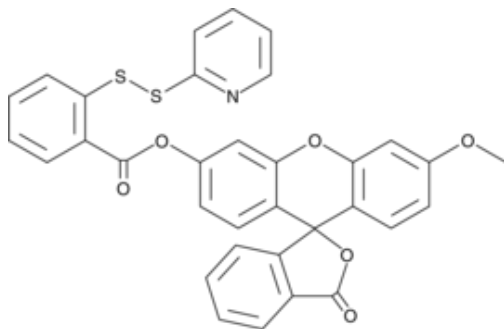
Formula C33H21NO6S2 M.Wt 591.7

Solubility 2mg/mL in DMSO, 20mg/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

WSP-1 Usage Instructions:

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

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Address: 10292 Central Ave. #205, Montclair, CA, USA

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Preparation of Stock Solution: Remove the product from the refrigerator and allow it to equilibrate at room temperature. Centrifuge at low speed for 3-5 minutes. Add an appropriate amount of anhydrous DMSO (Dimethyl Sulfoxide) to the vial and dissolve thoroughly to prepare a 5mM stock solution. Divide the solution into single-use aliquots and store them in the freezer, avoiding repeated freeze-thaw cycles. The stock solution should remain stable for at least three months.

For specific working concentrations, please refer to the literature or adjust as needed based on your experimental requirements.

Protocol for for Fluorescent Detection of Intracellular H₂S (Fluorescence Microscopy Examination) by WSP-1 [1]:

1. 1.Culture HASMCs until they reach approximately 90% confluence. 24 hours before the experiment, seed the cells on 8-well culture slides at a density of 30,000 cells per well and incubate for 24 hours.
2. 2.After 24 hours, allow the cells to adhere, replace the culture medium with 400 µL of WSP-1 (100 µM) working solution, and incubate at 37°C for 30 minutes.
3. 3.Replace the WSP-1 working solution with 380 µL of standard buffer and add 20 µL of the required concentration of H₂S donor compound.
4. 4.When WSP-1 reacts with hydrogen sulfide, a detectable fluorescence signal is released at Ex/Em = 465/515 nm. After incubating for 1 hour, wash the cells with 400 µL of DPBS.
5. 5.Remove the DPBS, and at room temperature, add 200 µL of Bouin's solution to each well to fix the cells for 10 minutes. Remove excess Bouin's solution and wash the cells twice with 400 µL of DPBS.
6. 6.Use a fluorescence microscope to examine and capture the fluorescence signals.

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Protocol for Visualizing Intracellular H₂S Using WSP-1 [2]:

1. 1.The roots of seedlings after treatments were transferred to 20 mM Hepes-NaOH (pH 7.5) buffer solution containing 15 μ M of WSP-1.
2. 2.After being incubated in darkness at 25°C for 40 min, the roots were washed with distilled water three times and were visualized immediately by a fluorescence microscope with a 465/515 nm and an excitation/emission filter set.
3. 3.For the detection of WSP-1 fluorescence in different reactive sulfur species, WSP-1 with final concentration of 15 μ M were added into the following solutions, SDS (sodium dodecyl sulfate, 2 mM), NaHSO₄ (2 mM), NaHSO₃ (2 mM), Na₂SO₄ (2 mM), Na₂SO₃ (2 mM), Na₂S₂O₄ (2 mM), GSSG (glutathione disulfide, 2 mM), sulfonamide (2 mM), GSNO (2 mM), and NaHS (2 mM), respectively. 20 μ L of the above solutions were transferred to a glass slide for the visualization with a fluorescence microscope with a 465/515 nm and an excitation/emission filter set.

This protocol only provides a guideline, and should be modified according to your specific needs.

References:

- [1]. Martelli A, Citi V, et.al. Vascular Effects of H₂S-Donors: Fluorimetric Detection of H₂S Generation and Ion Channel Activation in Human Aortic Smooth Muscle Cells. *Methods Mol Biol.* 2019;2007:79-87. doi: 10.1007/978-1-4939-9528-8_6. PMID: 31148107.
- [2]. Li YJ, Chen J, et.al. In site bioimaging of hydrogen sulfide uncovers its pivotal role in regulating nitric oxide-induced lateral root formation. *PLoS One.* 2014 Feb 27;9(2):e90340. PMID: 24587333

Background

WSP-1 is a selective and rapid responsive fluorescent probe for active sulfides, specifically designed for the detection of hydrogen sulfide (H₂S) within biological samples and intracellular environments. WSP-1 selectively and rapidly reacts with H₂S, generating benzodithiolone and a fluorescent compound (Ex=465nm, Em=515nm) [1].

WSP-1 (10 μ M) can detect sulfide levels in RFL-6 cells where the sulfide donor GYY4137 (10-100

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μM) is present[2]. The use of the WSP-1 fluorescent dye enables the detection of hydrogen sulfide (H_2S) generation in human vascular smooth muscle cells following H_2S donor administration[3]. WSP-1 can applied to track endogenous H_2S in tomato (*Solanum lycopersicum*) roots in site[4].

References:

- [1]. Liu C, Pan J, et,al. Capture and visualization of hydrogen sulfide by a fluorescent probe. Angew Chem Int Ed Engl. 2011 Oct 24;50(44):10327-9. doi: 10.1002/anie.201104305. Epub 2011 Sep 6. PMID: 21898737; PMCID: PMC3417056.
- [2]. Cortese-Krott MM, Fernandez BO, et,al. Nitrosopersulfide ($\text{SSNO}(-)$) accounts for sustained NO bioactivity of S-nitrosothiols following reaction with sulfide. Redox Biol. 2014 Jan 11;2:234-44. doi: 10.1016/j.redox.2013.12.031. PMID: 24494198; PMCID: PMC3909780.
- [3]. Martelli A, Citi V,et,al. Vascular Effects of H_2S -Donors: Fluorimetric Detection of H_2S Generation and Ion Channel Activation in Human Aortic Smooth Muscle Cells. Methods Mol Biol. 2019;2007:79-87. doi: 10.1007/978-1-4939-9528-8_6. PMID: 31148107.
- [4]. Li YJ, Chen J, et,al. In site bioimaging of hydrogen sulfide uncovers its pivotal role in regulating nitric oxide-induced lateral root formation. PLoS One. 2014 Feb 27;9(2):e90340. doi: 10.1371/journal.pone.0090340. PMID: 24587333; PMCID: PMC3937356.

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