

Product Data Sheet

Product Name: NSP-SA-NHS

Cat. No.: GC34691

Chemical Properties

Cas. No. 199293-83-9

SMILES O=S(CCC[N+](C)(C)C=C(C)C=C(C)C=C(C)C(N(CCCC(ON3C(CCC3=O)=O)=O)S(=O)(=O)C4=CC=C(C(C)C=C4)=O)=O)C5=C1C=CC=C5)([O-])=O

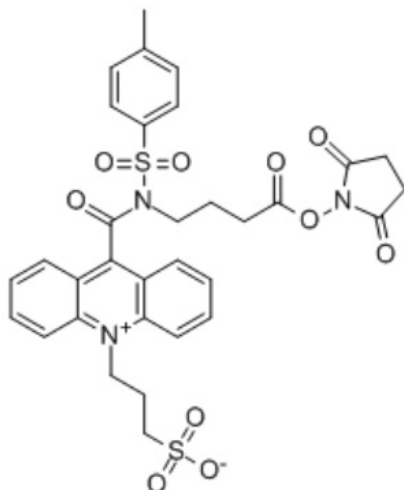
Formula $C_{32}H_{31}N_3O_{10}S_2$ M.Wt 681.73

Solubility DMSO : 11.36 mg/mL (16.66 mM; ultrasonic and warming and heat to 60°C) Storage Store at -20°C, sealed storage, away from moisture and 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

Synthesis and Analysis of the BVA-BSA-NSP-SA Complex [1] :

(1) Dissolve 1mg of BSA in 0.3mL of PBS (Solution A), and dissolve 0.2mg of NSP-SA-NHS in 40 µL of DMF (Solution B).

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(2) Slowly add Solution B to Solution A and shake (130r/min) for 12 hours under a light-protected condition at 18°C.

(3) The resulting solution is dialyzed with 500mL of distilled water at 4°C for 2 days, with the distilled water changed twice during the process to remove free NSP-SA-NHS, and the solution C is obtained.

(4) Precisely weigh 0.07mg of 4,4-bis(4-hydroxyphenyl)pentanoic acid (BVA), 0.14mg of NHS, and 0.2mg of 1-ethyl-3-[3-dimethylamino propyl] carbodiimide hydrochloride (EDAC), dissolve them in 30mL of DMF, and let the reaction proceed overnight at 17°C to obtain Solution D.

(5) Slowly add Solution D to Solution C and shake (130r/min) for 24 hours, then dialyze with distilled water for 48 hours. During this period, the distilled water is changed four times.

(6) Dilute the coupling agent to an appropriate concentration with distilled water and analyze it using a UV spectrophotometer.

Chemiluminescence analysis of Tau concentration [2]:

1. Preparation of conjugated detection antibodies:

(1) Dissolve NSP-SA-NHS in DMF to prepare a 5mM AE-NHS solution.

(2) Adjust the concentration of the uncoupled antibody (db8769, mouse polyclonal) to 1.0mg/mL using a 1M sodium bicarbonate aqueous solution (pH = 8.3), and add 5μL of the DMF solution containing NSP-SA-NHS. React at 30°C for approximately 30 minutes.

(3) After the reaction, the labeled antibody was purified using a gel chromatography column, resulting in an AE-conjugated detection antibody with a final concentration of 1μg/mL, which was stored at -20°C.

2. Chemiluminescence determination:

(1) Set up standard samples of different concentrations. Mix 100μL of the sample with biotin antibody (50μL) and AE-conjugated detection antibody (50μL) respectively, to form a sandwich complex specific to total Tau.

(2) After adding the avidin-coated superparamagnetic iron oxide nanoparticles, the sandwich complex

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binds to the solid substrate. Using a magnetic field, the solid phase and the liquid phase are separated.

(3) Use thorough rinsing with 0.1M PBS buffer (0.05% Tween 20, pH 7.4) to remove unbound unreacted antibodies and other biomolecules.

(4) By using sodium hydroxide (the triggering solution) as an intermediary, a photomultiplier tube can be employed to monitor and quantify the reduced current.

This agreement merely provides a guiding principle. When implementing it, adjustments should be made according to your actual needs.

References:

[1] Fan Fan Yang, et al. A Rapid and Sensitive Chemiluminescent Immunoassay of Bisphenol a with NSP-SA-NHS-Labeled. Applied Mechanics & Materials, 2014, 707:7-11.

[2] Zhang L, Wang D, Dai Y, et al. Machine Learning Reveals a Multipredictor Nomogram for Diagnosing the Alzheimer's Disease Based on Chemiluminescence Immunoassay for Total Tau in Plasma[J]. Frontiers in Aging Neuroscience, 2022, 14: 863673.

Background

NSP-SA-NHS is a chemiluminescent acridine ester compound with high stability, activity and sensitivity [1]. In alkaline H₂O₂ solution, the acridine ester is attacked by hydrogen peroxide ions, generating a tense and unstable diethylene oxide, which further decomposes into CO₂ and electronically excited-state acridine ketone. When the acridine ketone returns to the ground state, it emits photons with a maximum absorption wavelength of 430nm [2]. NSP-SA-NHS can be used in chemiluminescence and immunoanalysis, receptor analysis, nucleic acid and peptide detection and other studies [3].

Product characteristics:

1. Before the formation of the electronically excited-state intermediate, the non-luminescent substituent part connected to the acridine ring separates from the luminescent part, so the luminescence efficiency is basically not affected by the substituent structure.
2. No catalyst or enhancer is required. It can emit light in a dilute alkaline solution with H₂O₂.

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3. Its luminescence type is flash type. The luminescence intensity reaches the maximum in about 0.4s after adding the excitation solution, and the half-life is about 0.9s.

Product stability:

1. It is stable in acidic solutions ($\text{pH} < 4.8$). When coupled with protein conjugates and stored at room temperature for 4 weeks, the light quantum yield does not decrease. The freeze-dried product can be stored for more than one year at -20°C .
2. When $\text{pH} > 4.8$, especially in alkaline solutions, the acridine compound has reduced stability due to partial hydrolysis. The hydrolysis process is an inactive dark reaction process. The degree of hydrolysis increases with pH increase and temperature increase.
3. The stability of acridine amide is higher than that of acridine ester structure, and has a stronger anti-hydrolysis ability.
4. When there are electronegative groups such as methyl groups attached to the acridine ring, phenol ring or benzenesulfonamide ring, due to the large steric hindrance, the thermal stability increases; when there are electron-withdrawing groups, it is conducive to nucleophilic substitution reactions and its stability decreases.
5. If there is a decrease in luminescence after protein labeling, it is recommended:

The buffer solution used for storage after coupling should be as weakly acidic as possible. Blow nitrogen to remove the oxygen in it. Seal and store in a dark place at low temperature. If conditions permit, it can be made into freeze-dried for storage.

References:

- [1] Yang F, Xu L, Liu X, et al. Chemiluminescence immunoassay approach to quantify Bisphenol S in canned beverage using a NSP-SA-labeled specific monoclonal antibody[J]. European Food Research and Technology, 2020, 246(9): 1857-1865.
- [2] Zhang L, Wang D, Dai Y, et al. Machine Learning Reveals a Multipredictor Nomogram for Diagnosing the Alzheimer's Disease Based on Chemiluminescence Immunoassay for Total Tau in Plasma[J]. Frontiers in Aging Neuroscience, 2022, 14: 863673.
- [3] Du D, Wang J, Guo M, et al. Charge-dependent signal changes for label-free electrochemiluminescence immunoassays[J]. Analytical Chemistry, 2022, 94(47): 16436-16442.

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