

Product Data Sheet

I. Protocol for Synthesis of albumin conjugates and NanoAlb-proDOX conjugate using SPDP [1]:

1. HSA was dissolved in PBS-EDTA buffer (20mM sodium phosphate, 150mM NaCl, 1mM EDTA, pH 7.5) to a concentration of 200 μ M.
 2. SPDP stock solution was prepared in DMSO. To initiate the reaction, 50 μ M HSA and 1mM SPDP were gently shaken for 2h at room temperature in PBS-EDTA buffer.
 3. After the reaction, the excess SPDP was removed by flowing through a PD-10 column.
 4. Then, 23mg/ml DTT in acetate buffer (100mM sodium acetate buffer, 100mM NaCl, pH 4.5) was added to SPDP-labeled HSA in PBS-EDTA buffer with a final concentration of HSA of approximately 100 μ M (the ratio of acetate buffer and PBS-EDTA buffer was kept at 1:2 to keep the pH of the reaction system relatively acidic, preventing the native and intracellular disulfide bonds from being affected by DTT reduction).
 5. The reaction proceeded at room temperature for 1h, and the excess DTT was removed by flowing through a PD-10 column.
 6. The reduced HSA solution was then allowed to react with 100 μ M aldoxorubicin (AIDOX, prediluted with 20% of total reaction volume of DMSO) with a final HSA concentration of 20 μ M in PBS-EDTA buffer. The mixture was gently shaken overnight at room temperature.
 7. Finally, excess AIDOX was removed by flowing through a PD-10 column.
- NanoAlb-proDOX solution was stored in PBS buffer and preserved at -80°C .

II. Protocol for In Vivo Cross-Linking Using SPDP [2]:

1. Cultures were grown to OD600 0.8.
2. The sample was washed twice, concentrated 7.5-fold with cross-linking buffer (20mM Na-phosphate, pH 7.5 /150mM NaCl /1mM EDTA) and incubated for 30min with either DMSO only, 0.2mM SPDP, or 0.2mM LC-SPDP each in DMSO.
3. After quenching with 2.5mM Tris-HCl (pH 7.4), cells were harvested and washed twice with cross-link buffer.
4. Cell membranes were solubilized in 8M urea, 1% Triton X-100, 20mM Na-phosphate (pH 7.5).
5. Complexes were affinity-purified from solubilized membranes by incubating for 1h with 50 μ l Ni-NTA resin and washed, first with 10mM imidazole, 8M urea, 1% Triton X-100, 20mM Na-phosphate (pH 7.0), then with 20mM imidazole, 1% Triton X-100, 20mM Na-phosphate (pH 7.0), 0.5M NaCl, 0.1% SDS, and third with 30mM imidazole, 1% Triton X-100, 20mM Na-phosphate (pH 7.5), 150mM NaCl, 20% glycerol. All washes were carried out three times each.
6. Proteins were eluted with 8M urea, 50mM Tris-HCl, 2% SDS, 0.4M imidazole (pH 6.8), and

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cleaved from the complex by reducing the cross-linker with 100mM DTT (30 min at 37°C), resolved by SDS/10% PAGE.

References:

- [1]. Chen L, Xu N, et.al. Nanoalbumin-prodrug conjugates prepared via a thiolation-and-conjugation method improve cancer chemotherapy and immune checkpoint blockade therapy by promoting CD8+ T-cell infiltration. *Bioeng Transl Med*. 2022 Jul 30;8(1):e10377. doi: 10.1002/btm2.10377. PMID: 36684090; PMCID: PMC9842047.
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Background

SPDP (SPDP Crosslinker) is a short-chain crosslinker that reacts with NHS ester groups and pyridyl disulfide reactive groups with cysteine thiols to form a cleavable (reducible) disulfide bond, thereby facilitating the coupling of amine and thiol groups. When SPDP reacts with free thiols, it releases a detectable byproduct—2-mercaptopyridine—allowing for easy tracking of the reaction by measuring the release of 2-mercaptopyridine at 343 nm. SPDP can be used for the synthesis of antibody-drug conjugates. The SPDP crosslinker is membrane-permeable and can crosslink within cells [1-6].

References:

- [1]. Singh V, Mavila AK, et.al. Effect of lysine residue modification of ovine luteinizing hormone by heterobifunctional crosslinking reagent SPDP on subunit-subunit association, receptor binding and biological activity. *Indian J Exp Biol*. 1992 Nov;30(11):1093-100. PMID: 1284055.
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[6]. Zhao H, Li L, Peng Z, et,al. Improved targeting delivery of WED-load immunoliposomes modified with SP-A mAb for the treatment of pulmonary fibrosis. Colloids Surf B Biointerfaces. 2023 Apr;224:113237. doi: 10.1016/j.colsurfb.2023.113237. Epub 2023 Mar 1. PMID: 36871414.

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