Instructions for SPDP-PEG Conjugation

1.Introduction

SPDP-PEG cross-linking agent can achieve protein coupling through amine-amine or amine-mercapto cross-linking. Before adding the reaction mixture, the SPDP-PEG cross-linking agent is preferably dissolved in an organic solvent. The SPDP reagent will generate disulfide-containing bonds that can be cleaved with reducing agents such as dithiothreitol (DTT).

The amine-reactive part of the SPDP reagent is n-hydroxysuccinimide (nhs) ester. The coupling reaction was performed by NHS ester in a pH of 7-8, without a primary amine.

The sulfhydryl reaction part of SPDP reagent is 2-pyridyldithiol, and the best reaction is in the pH 7-8 sulfhydryl free buffer. By measuring the absorbance at 343 nm, the results of the pyridine-2-thione displacement reaction can be determined.

The obtained crosslinked product contains disulfide and can be reduced and cracked by dithiothreitol (DTT) or THPP and TCEP. In most cases, crosslinks generated using SPDP reagents can be cleaved with 25mM DTT at pH 4.5 without need to reduce
the disulfide bonds of natural protein.

2.Process and store information

- After receiving the goods, please store them in a moisture-proof place at -20 ° C.
- The product is transported in a sealed bag with a desiccant at room temperature. In addition, the bottle is wrapped with laboratory film to protect the product from moisture and maintain product integrity.

3.General steps of protein-SPDP-PEG coupling

1. Dissolve 5 mg of SPDP-PEG in 640 µL of DMSO or DMF to obtain a 25 mM stock solution.
2. Dissolve amine-containing protein A at a concentration of 1-5 mg / ml in 100 mm sodium phosphate buffer (pH 7.2 to pH 8.0, 1 mm EDTA).
3. Add 20 µL of 25 mM SPDP-PEG solution to 1 mL of the above protein A solution.
4. Allow the reaction to continue at room temperature for 30-60 minutes.
5. Remove unreacted SPDP crosslinker by gel filtration.
6. Dissolve thiol-containing protein B in buffer (100 mM sodium phosphate, pH 7.2 to 8.0, 1 mM EDTA).
7. Add 0.2 to 1.0 molar equivalent of protein B solution to desalted activated protein A.
8. The reaction is allowed to proceed at room temperature for 8 to 16 hours.