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A Geno Technology, Inc. (USA) brand name

DSS, OneQuant™ DSS

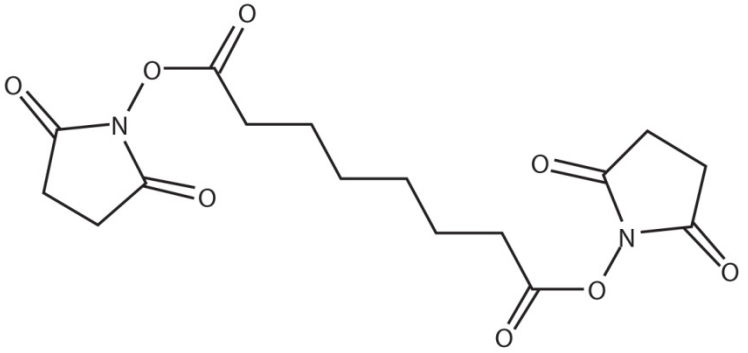
Disuccinimidyl suberate

(Cat. # BC04, BC04-Q)

INTRODUCTION

DSS (Disuccinimidyl suberate) is a water insoluble, homobifunctional cross-linker that reacts with primary amines through its two NHS (*N*-hydroxysuccinimide)ester reactive groups. The efficient reaction occurs in pH7-9 buffers to form stable amide bonds, releasing *N*-hydroxysuccinimide.

DSS is water insoluble, hydrophobic chemical and must be dissolved in an organic solvent, such as DMSO or DMF and then added to the aqueous reactions. An advantage of DSS is that is has no charged groups and is membrane permeable, which makes it ideal for intracellular and intra-membrane conjugations.



SPECIFICATIONS

- **CAS #:** 68528-80-3
- **Molecular Weight:** 368.4
- **Spacer Arm (Å):** 11.4
- **Reactive Toward:** Primary Amines
- **Membrane Permeable:** YES
- **Water Soluble:** NO
- **Cleavable/ Reversible:** NO

ITEM(S) SUPPLIED

Cat. #	Description	Size
BC04	DSS	1g
BC04-Q	DSS, OneQuant™	8 x 2mg

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival store desiccated at 4°C. Stable for 1 year when stored and used as recommended.

ADDITIONAL ITEM(S) REQUIRED

DMSO (Cat. # BKC-17) or DMF (Cat. # BKC-16)

IMPORTANT INFORMATION

- DSS is moisture sensitive. Allow to warm to room temperature before opening vials to prevent condensation formation. Store with a suitable desiccant.
- Prepare DSS immediately before use. The NHS ester reactive group readily hydrolyses becoming non-reactive. Do not prepare stock solutions and discard any unused reagents.
- Avoid buffers containing primary amines, such as Tris or glycine.

PREPARATION BEFORE USE

For DSS, allow vial to equilibrate to room temperate and weigh 2mg into a suitable vial. Add the recommended volume of DMSO or DMF.

For OneQuant™ DSS, allow vials to equilibrate to room temperature. If only using 1-2 vials then cut vials from strip and return unused vials to correct storage conditions. Pierce the foil with a pipette tip and add the recommended volume of DMSO or DMF.

Solvent Volume for 2mg DSS	DSS Concentration
434µl*	12.5mM
217µl	25mM
109µl	50mM
54µl	100mM

*OneQuant™ tubes have a maximum volume of 220µl.

PROTOCOL: CROSSLINKING PROTEINS

Additional Item(s) Required

- DMSO or DMF
- Coupling Buffer (0.01M sodium phosphate, 0.15M NaCl; pH 7.2)
- Quenching Buffer: 51M Tris.HCl, pH7.5

Procedure

1. Prepare protein sample in Coupling Buffer. If protein is in a buffer containing primary amines (Tris or glycine) then dialyze against Coupling Buffer.
2. Prepare DSS immediately before use as described above.
3. Add DSS to the protein solution, using a 10-fold molar excess for >5mg/ml protein concentration and a 20 to 50-fold excess for samples with <5mg/ml protein concentration. The final concentration should be 0.25-5mM.
4. Incubate at room temperature for 30 minutes or on ice for 2 hours.
5. Quench the reaction with a final concentration of 50mM Tris for 15 minutes or remove excess DSS by dialysis or desalting.

PROTOCOL: CROSSLINKING FOR IMMUNOPRECIPITATIONS

Traditional immunoprecipitations use and antibody bound to an antibody capture protein, such as Protein A, G or A/G, however some elution steps result in the release of the antibody and contamination of your sample. To overcome this, DSS is used to covalently couple the antibody to the antibody capture protein.

Additional Item(s) Required

- Immobilized Protein A, G or A/G Resin (Cat. # 786-283, 786-829, 786-836)
- Primary Antibody
- DMSO or DMF
- Coupling Buffer (0.01M sodium phosphate, 0.15M NaCl; pH 7.2)
- Elution Buffer: 50mM Glycine, pH2-3

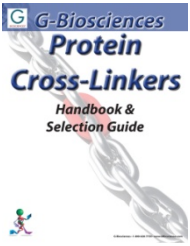
Procedure

NOTE: *The cross-linking step is designed for 10µg antibody, but is suitable for 2-50µg.*

1. Equilibrate 10µl Immobilized Protein A, G or A/G in coupling buffer by washing with 3 x 200µl Coupling Buffer.
2. Prepare 10µg affinity purified antibody by adjusting the volume to 100µl with Coupling Buffer. Add directly to the resin in the tube.
3. Incubate at room temperature for 0.5-1 hour with end-over-end mixing.
4. Centrifuge for 60 seconds at 1,000-2,000xg and remove the antibody solution and save the supernatant to determine antibody coupling.
5. Add 500µl Coupling Buffer to the resin, centrifuge for 60 seconds at 1,000-2,000xg and discard the supernatant. Repeat wash step one more time.
6. Add 100µl dry DMSO to a vial of OneQuant™ DSS and pipette up and down to dissolve. Dilute this solution by adding 50µl to 950µl DMSO.
7. Add 10µl Coupling Buffer, 9µl diluted DSS solution and 31µl distilled water directly to the resin.
8. Incubate at room temperature for 0.5-1 hour with end-over-end mixing.
9. Wash the resin with 300µl Elution Buffer. Repeat this step two more times
10. The antibody is now covalently coupled to the Imobilized ProteinA, G or A/G resin and is ready for immunoprecipitation experiments.

RELATED PRODUCTS

Download our Protein Cross-Linker Handbook.



http://info.gbiosciences.com/complete-protein-cross-linkers-handbook

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