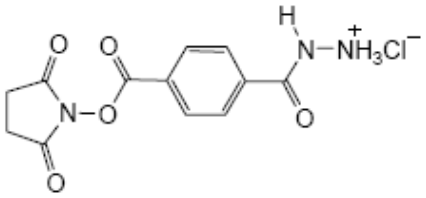
FT-BL9370

SHTH

Product Information

To modify biomolecules or surfaces with stable hydrazide-reactive moities

Catalog #: BL9370, 10 mg
Name: SHTH
Succinimidyl 4-hydrazidoterephtalate hydrochloride
MW 313.69
Spacer Arm 7.9 A
Storage: <-20°C
Dessicated under inert atmosphere



Applications:

Incorporation of aromatic hydrazide moieties on proteins or other amine containing moieties.
SHTH is most widely used to produce a variety of antibody-related conjugated such as IgG-oligo. Conjugates of other antibody fragments have also been made. SHTH is also used for the synthesis of numerous other conjugates including: general protein-protein, protein-peptide, peptide-oligonucleotide, enzyme-oligonucleotide and DNA or RNA-protein conjugates.

Introduction

SHTH (succinimidyl 4-hydrazidoterephtalate hydrochloride) is available to incorporate 4-hydrazidoterephtalate moieties on biomolecules and surfaces by using the Couplage Bioconjugation System. The SHTH chemistry converts amine groups to aromatic hydrazide groups that can form stable bis-aryl hydrazide hydrazones. The SHTH chemistry yields an even more stable modified biomolecule than the SANH chemistry. Couplage is a novel bioconjugation system using the HydraLink technology for the conjugation and immobilization of peptides, proteins, carbohydrates and DNA/RNA. The conjugation chemistry is simple to perform, stable in solution, highly selective for heteroconjugation, and does not lead to non-specific conjugation. Moreover, biomolecules with reactive moieties can be prepared ahead of time, stored, and used as needed for subsequent conjugations, making the SHTH technology superior to current methods of bioconjugation such as maleimide/thiol and avidin/biotin.

The conjugate bond is stable to 92OC and pH 2.0-10.0. The recommended pH for antibody conjugation is 6.0.

Directions for use

Protocol 1 (r)

A. Materials Required

• Modification Buffer: 100 mM phosphate, 150 mM sodium chloride, pH 7.2-7.4 (PBS, #UP68723A)

• Conjugation Buffer: The optimal pH for hydrazone formation is 4.7; however, conjugation proceeds in any non-amine containing buffer up to pH 7.2, with slower kinetics. Suggested Conjugation Buffers are as follows:

100 mM MES, 150 mM NaCl; pH 4.7 (MES Buffer, #GS2960)
100 mM citrate, 150 mM NaCl; pH 6.0
100 mM phosphate, 150 mM NaCl; pH 7.2 (PBS, #UP68723A)

• Desalting column, 2 ml (#BI7740), or a dialysis unit (#AA7411)

• Dimethylformamide (DMF, #121370) or dimethylsulfoxide (DMSO, #FP-JW7390)

• Protein assay kit such as the BC Assay (#UP40840A) or the Coo Protein Bradford Assay Reagent (#UPF86420)

B. Protein Preparation

Dialyze proteins against the Modification Buffer and determine concentration. For best results use proteins at ≥ 1 mg/ml for the modification reactions.

C. Protein Modification

This protocol is for modifying proteins using either the hydrazine/hydrazide reagents (i.e., SANH, C6-SANH and SHTH) or the aldehyde reagents (i.e., SFB and C6-SFB). Therefore, perform this protocol twice; for example, modify the first protein with SANH, C6-SANH or SHTH and then modify the second protein with SFB or C6-SFB.

1. Immediately before use, dissolve 2.0-4.0 mg of modification reagent in 100-200 µl DMF or DMSO.
2. Add a volume of modification solution to the protein to achieve a 10- to 20-fold molar excess of the reagent.To minimize damage to the protein, do not exceed 10% solvent in the final reaction mixture. For optimal results, use < 5% solvent in the reaction.
3. Incubate reaction at room temperature for 2-3 hours. Proceed to protein conjugation or store modified proteins at 4°C.

Hydrazine moieties (i.e., SANH and C6-SANH) are stable for several months. Hydrazide (i.e., SHTH) and aldehyde (i.e., SFB and C6-SFB) moieties are stable indefinitely.

D. Protein Conjugation

Conjugation efficiency is dependent on protein modification level, buffer composition and pH, protein concentration and reaction time. Hydrazone formation is an acid-catalyzed reaction with an optimal pH of 4.7, but the reaction proceeds in conditions up to pH 7.2 with slower kinetics. Generally, protein concentrations ≥ 0.1 mg/ml will produce sufficient conjugates.

1. Desalt or dialyze the modified proteins against the Conjugation Buffer to remove excess reagent and to exchange the buffer.
2. Determine protein concentration using the BC Protein Assay or the Coo Protein Bradford Assay Reagent. The modification moieties significantly contribute to the extinction coefficient of the protein; the refore, do not use absorbance measurements to determine protein concentration.
3. Combine the desired amount of each modified protein and incubate. At pH 4.7-5.0 incubate reaction for 1-3 hours. At pH 7.0-7.2 incubate the reaction for > 6 hours. To evaluate conjugation progression, perform SDS-PAGE analysis on a portion of the reaction mixture.
4. To isolate conjugate, use size exclusion chromatography or ion exchange chromatography.

Related products

- Controled Amine Conjugation kit (SANH/SFB), BL1501
- ControledAmine Conjugation Kit (SHTH/SFB), BL1521
- Succinimidyl 4-FormylBenzoate (SFB), M11771
- C6-SFB, BL9410
- Sulfo-SFB, BI1311

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06