FT-G9906A

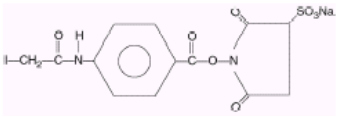
SIAB, Sulfo-SIAB, SBA, SBAP
Heterobifunctional Amine/Sulfhydryl cross-linkers

Description

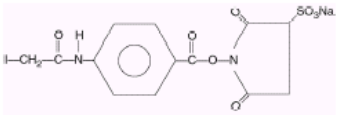
Catalog number: 92177A, 50mg 92177C, 1g
Name: **SIA**
Formula : N-Succinimidy-iodoacetate
CAS:[39028-27-8], M.W.= **283.02**

Spacer Length: 1.5 Å

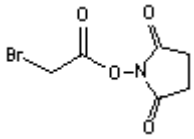
Catalog number: G9906A, 100mg G9906B, 50mg
Name: **SIAB**
Formula : N-Succinimidyl(4-iodoacetyl)aminobenzoate
CAS:[72252-96-1], M.W.= **402.15**



Catalog number: 75036A, 100mg 75036B, 50 mg
Name: **Sulfo-SIAB**
Formula : Sulfosuccinimidyl(4-iodoacetyl)aminobenzoate
CAS:[144650-93-1], M.W.= **504.2**



Catalog number: 69380A, 100mg 69380B, 50mg
Name: **NHS-BromoAcetate, SBA**
Formula : Succinimidyl-Bromoacetate ; Bromoacetic acid.
M.W.=**236.03**



Catalog number: L7737A, 100mg
Name: **SBAP**
Formula : Succinimidyl 3-[bromoacetamido]propionate
M.W.=**307.10**

NHS group reacts with amines at pH7-9, while bromoacetyl group reacts with sulfhydryl at pH>7.5 forming an amide bond and a thioeter bound, with a peptide-like spacer, susceptible to acid hydrolysis.

Storage : SIA, SIAB: -20°C, protect from moisture and Light. (M)
Sulfo–SIAB :+4°C (possible at –20°C), protect from moisture and light (L)

Introduction

SIAB and Sulfo-SIAB are crosslinkers with amine-reactivity (through a succinimidyl group) and sulfhydryl-reactivity (through a iodoacetyl group), providing a cross-bridge with 10.6 Å spacer between conjugated molecules. These heterobifunctional crosslinkers are used to prepare a variety of conjugates, including enzyme conjugates.
SBAP has been used for preparing cyclic peptides and peptide conjugates because the spacer maintains peptide-like character in the crosslinked species.

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Guidelines for Use

The following standard protocol uses a first step to activate IgG by SIAB (NHS reaction of amino groups) , and a second step to react with free sulfhydryls present on the surface of native β-galactosidase. The ratio of IgG to β-galactosidase should be optimized depending on each application.

0. Prepare reagents & materials:

- Borate buffer: 50 mM sodium borate, pH 8.5 (#GS2950), 5 mM EDTA (#UP03629)
- 1 mg/ml IgG in borate buffer (use i.e. dialysis, gelfiltration or ultrafiltration) (quantitate proteins with BC Assay #UP48084 or Bradford Assay #UPF8640)
- Prepare desalting tools: equilibrate Desalt Spin Columns (#UP84874), or CelluSep dialysis tubings
- Cysteine•HCl (#GS2970)

1. Just before use, dissolve 1.4 mg SIAB in 1 ml DMSO or dissolve 1.7 mg Sulfo-SIAB in 1 ml of ultrapure water. Protect solutions from light.

2. Add 10 µl of cross-linker solution to 1 ml of IgG and react for 30 minutes at room temperature.

3. Remove non-reacted cross-linker using a desalting column equilibrated with borate buffer.

4. Add 4 mg of β-galactosidase to the desalted IgG and react for 1 hour at room temperature in the dark.

5. To quench the reaction, add a final concentration of 5 mM cysteine and react for 15 minutes at room temperature in the dark.

6. Remove non-reacted reagents by desalting or dialysis.

Scientific and Technical Information

The Sulfo-NHS group of Sulfo-SIAB makes this crosslinker water-soluble and membrane-impermeable, while SIAB is water-insoluble and therefore membrane-permeable. Sulfo-SIAB is soluble up to a concentration of approximately 10 mM in aqueous buffers. SIAB must first be dissolved in an organic solvent and then aliquoted into the aqueous reaction mixture. The most commonly used organic solvents for this purpose are dry DMSO or DMF. Note that SIAB is not stable in DMSO or DMF from a practical standpoint because these solvents are very hygroscopic. These solvents tend to absorb moisture and thus promote hydrolysis of the NHS portion of SIAB. We do not recommend preparing stock solutions of either SIAB or Sulfo-SIAB with the intent of long-term storage, since the NHS and Sulfo-NHS esters readily hydrolyse in water.

The **amine-reactivity** is provided by the chemical group N-hydroxysuccinimydyl, NHS or Sulfo-NHS as esters. NHS reacts in aqueous phase on primary ($-NH_2$) and secondary amines (=NH) (in fact on its deprotonated form), optimally at neutral pH or higher : amines present in proteins (Lys aminoacid) and in a lower proportion on NH_2 located in terminal peptidic chains. The reaction can take place in a range of conditions, with incubation temperatures of 4-37°C, reaction mixture pH values of 7-9, and incubation times ranging on the order of a few minutes to overnight. The reaction competes with hydrolysis that increases with pH, and with the high dilutions of the molecule that should be biotinylated. Amine containing buffers should be precluded (i.e. Tris, Glycine).

Amines are found in biomolecules, especially ubiquitous in all proteins (in lysine, a major amino-acids and are usually easily accessible on a protein's surface), or introduced by chemistry, as in aminated nucleotides. For site-directed conjugations, amines can be grafted chemically, or blocked with SulfoNHS-acetate (UP69380A).

The **sulfhydryl reactivity** of SIAB and Sulfo-SIAB is provided by an ***iodoacetyl*** group – the ***bromoacetyl*** group of SBA and SBAP is similar-. The reaction with the free sulfhydryl proceeds by nucleophilic substitution of iodine with a thiol group, forming a stable thioether linkage. The reaction can occur with other groups, but the specificity of reaction with sulfhydryls can be driven using slight stoichiometric excess of iodoacetyl groups over the present number of free sulfhydryls, and by keeping a pH 7.5 and 8.5 (optimally at pH8.3) for the reaction. If there is a gross excess of iodoacetyl group over the number of free sulfhydryls (or absence of free sulfhydryls), the iodoacetyl group can cross-react with amino acids. I.e. reaction occurs with Imidazoles at pH 6.9-7.0, for over a week [r]. Histidyl side chains and amino groups (unprotonated form) react with iodoacetyl groups above pH 5 and pH 7, respectively [r]. The iodoacetyl group is much more stable to hydrolysis as compared to the ester, so this reaction is usually performed in second step for conjugations in aqueous buffers. Reducing agents containing buffers should be precluded (i.e. DTT, mercaptoethylamine). Finally, the iodoacetyl reactions should be

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carried out in the dark to limit the generation of free iodine, which has the potential for reacting with tyrosine, histidine, and tryptophan amino-acids [r].

The sulhydryl reactivity of SBA is provided by the ***bromo*** group. It was found useful for preparing cyclic peptides, peptide conjugates, and polymers (Inman 1991).

Sulfhydryls are available naturally on the biomolecule2 to conjugate, usually in cystein residues of proteins, or introduced by chemistry as in peptides (often synthetized with a N-terminus Cysteine). Note that they can be oxidized (i.e. forming dissulfide bridges), thus non-reactive for conjugation, and often involved in protein function. When free sulfhydryls are not present in sufficient quantities on the second molecule, they can be introduced with Traut's Reagent (2-iminothiolane, UP42425A) or *N*-succinimidyl *S*-acetylthioacetate (SATA, UP84235A) ny reaction on amides. Alternatively, they can be generated through reduction of dissulfides with mercaptoethylamine•HCl., or DTT (UP284250), or TCEP (UP242214).

SIAB and SBA **conjugations** involves typically two steps: 1/acylation of amines from protein1 by NHS-ester 2/acylation of sulfhydryls from proteins by iodoactetyl or bromoacetyl group.
This two-step strategy is advantageous because a/ the hydrolysis is minimized, a NHS-ester is less stable b) the potential for excessive polymerization (among the proteins to be conjugated) is limited. Therefore, step1 lead to make an iodoacetyl-activated protein. The activated protein is then desalted and reacted in a second step with the sulfhydryl-containing protein.

In case the conjugate do not keep the expected properties, one should consider the possibility that sulfhydryls or amines used for the reaction were involved to protein's activity. Alternative strategies should be considered, as modifying amine or sulhydryls of one or 2 molecules and conjugating biomolecule2 to biomolecule1, or changing of crosslinking method (ask Uptima, i.e. Hydralink technology, carbohydrate conjugation to preserves the antigen-binding site of the immunoglobulin,...).

The optimal preparation of a conjugate is largely dependent on the ratio of coupled biomolecules, determined primarily by the degree of activation in step1 (number of iodoacetyl groups incubated then attached per biomolecule1). The concentration of reagents should be adjusted depending on the number of amines (and sulfhydryls) on each biomolecules, biomolecules concentration, reactions yield.... Check their level with OPA (UP284250) and HABA (UP05361D) reagents. Now, on should be aware of eventual variations of reactive groups number in each biomolecule population. Finally, calibration is required to yield optimal results for each protein conjugation.

References:

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Other information

For research use only.

Related products:

*Other crosslinkers :
▪Heterobifunctional crosslinkers: Maleimido-NHS reagents (i.e. SMCC), photoActivable / amine reactive (i.e. SCBP #BI1361),...
▪Biotin Labeling : NHS-Biotins i.e. NHS-PEO4-Biotin UPR2027A, Maleimide-Biotins
▪Fluorescent Labeling : FluoProbes chromophores labeling agents (NHS, Maleimide, Hydrazide...)
*Desalting tools: ▪CelluSep dialysis tubings ▪Desalting gelfiltration columns #UP84874
*Carrier proteins: ▪KLH UP88502, BSA, OVA MaxiBind proteins

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