INSTRUCTIONS

SIA

22349

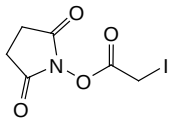
0790.2

Number	Description
22349	**SIA** (*N*-succinimidyl iodoacetate), 50mg

Formula: $C_6H_6INO_4$

Spacer arm: 1.5Å

Molecular weight: 283.02



Storage: Upon receipt store at 4°C protected from moisture. Product is shipped at ambient temperature.

Introduction

Thermo Scientific SIA is the shortest sulfhydryl-reactive and amine-reactive heterobifunctional crosslinker available and is often used for preparing enzyme conjugates or immunotoxins. This crosslinker contains an amine-reactive *N*-hydroxysuccimide (NHS) ester and a sulfhyryl-reactive iodoacetyl group. NHS esters react with primary amino groups ($-NH_2$) present on the side chain of lysine (K) residues and the N-terminus of polypeptides. Iodoacetyl groups react with free sulfhydryls by nucleophilic substitution of iodine with a thiol group, resulting in a stable thioether linkage.

Product Information

- SIA is moisture-sensitive. To avoid moisture condensation onto the product, vial must be equilibrated to room temperature before opening.
- Prepare this crosslinker immediately before use. The NHS-ester moiety readily hydrolyzes and becomes non-reactive; therefore, do not prepare stock solutions for storage. Discard any unused reconstituted crosslinker.
- The amine-reactive NHS ester couples to amines in 0.1M sodium phosphate, 0.15M sodium chloride, pH 7.2. Avoid amine-containing buffers, such as Tris or glycine, because they will compete with the NHS-ester reaction. Reactions are typically complete in 30-60 minutes at room temperature or within 2 hours at 4°C.

Protocol for Conjugating IgG to β-Galactosidase

In this two-step method, an iodoacetyl-activated IgG is prepared first. The activated IgG is then reacted with free sulfhydryls present on the surface of native β-galactosidase. Optimize the ratio of IgG to β-galactosidase as needed.

Materials Required

- Borate buffer (50mM sodium borate, pH 8.3, 5mM EDTA)
- Phosphate-buffered saline (PBS; 10mM sodium phosphate, 150mM sodium chloride, pH 7.4)
- IgG prepared at 1mg/mL in borate buffer
- Dextran desalting column with a 5mL bed volume (Product No. 43230)
- β-Galactosidase

  Note: Do not use β-galactosidase preparations that contain reducing agents, such as β-mercaptoethanol, because this will quench the reaction.
- Thermo Scientific Coomassie Protein Assay Reagent (Product No. 23200)
- Cysteine•HCl (Product No. 44889) for quenching non-reacted iodoacetyl groups

Protocol

1. Just before use, dissolve 1.4mg of SIA in 1mL DMSO. Protect solution from light.
2. Add 10µL of crosslinker solution to 1mL of IgG and react for 30 minutes at room temperature.
3. Equilibrate a desalting column with borate buffer and apply the reacted IgG sample to the column. Add borate buffer to the column and collect 0.5mL fractions.
4. Monitor the fractions with Coomassie Protein Assay Reagent and pool IgG-containing fractions.
5. Add 4mg of β-galactosidase to the IgG and react for 1 hour at room temperature in the dark.
6. Add cysteine to a final concentration of 5mM and react for 15 minutes at room temperature in the dark.
7. Equilibrate a desalting column with borate buffer and apply the conjugated sample to the column. Add borate buffer and collect 0.5mL fractions.
8. Monitor protein content of the fractions with Coomassie Protein Assay Reagent and pool the conjugate-containing fractions.

Related Thermo Scientific Products

20036 **Bioconjugate Techniques, 2nd Edition,** softcover

28384 **BupH™ Borate Buffer Packs,** 40 packs

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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