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A Geno Technology, Inc. (USA) brand name

ActiveHOOK™ BSA

(Cat. # 786-087)

INTRODUCTION

ActiveHOOK™ BSA is a pre-activated form of immunological grade BSA (MW 67kD) that has been activated by sulfoSMCC and is ready for direct coupling with peptides through the cysteine residues. Immunological grade BSA is a high purity grade bovine serum albumin (BSA) prepared for immunotechnology research applications such as a carrier protein for antibody production. ActiveHOOK™ BSA is supplied lyophilized in 10mg/vial size.

ITEM(S) SUPPLIED (Cat. # 786-087)

Description	Size
ActiveHOOK™ BSA	10mg

STORAGE CONDITION

Shipped at ambient temperature. Upon receiving, store it at 4°C.

IMPORTANT INFORMATION

1. ActiveHOOK™ BSA contains 15-25 moles of maleimide per mole of BSA.
2. Use a molar excess of peptide over the carrier protein's maleimide groups to ensure complete and efficient conjugation. For example, if the peptide's molecular weight is 2kD, then add 2mg (1µmol) to 2mg of carrier protein (~0.7µmol of maleimide groups). Alternatively, if a molar excess of peptide is not available, after conjugation, add a sulfhydryl containing compound, such as cysteine, to quench any remaining active maleimide groups.
3. For peptides insoluble in Coupling Buffer, we recommend the use of DMSO (<30%).
4. Conjugation efficiency will vary from differences in the size and structure of peptides. The protocol is designed for the widest variety of applications, but is not necessarily optimal for a specific peptide. It may be possible to use less peptide and still obtain good results.
5. Maleimides react with free sulfhydryls to form stable thioether bonds at pH 6.5-7.5. pH >7.5 significantly increases the reaction of amines with the maleimide groups.
6. Some sulfhydryl-containing peptides and proteins may oxidize in solution and form disulfide bonds, which cannot react with maleimides. Disulfide bonds can be reduced to produce free sulfhydryls. The G-Biosciences Immobilized Reductant (Cat. # 786-148) enable peptide or protein reduction while recovering the sample in the absence of reducing agents. In addition EDTA is included in the Coupling Buffer to prevent metal-catalyzed oxidation of sulfhydryls.
7. Ellman's Reagent (Cat. # BC87) can be used to determine the amount of free sulfhydryls. (See Appendix)
8. For peptides or proteins lacking sulfhydryls, SATA (N-Succinimidyl-S-acetylthioacetate) (Cat. # BC96) or Traut's Reagent (2-Iminothiolane hydrochloride) (Cat. # BC95) can be used to add sulfhydryls via amine modification. (See Appendix)

ADDITIONAL ITEM(S) REQUIRED

- Peptide: 2mg of peptide of choice to be coupled.
- Coupling Buffer: A physiological pH phosphate buffer (0.1M sodium phosphate, 0.15M NaCl, 0.1M EDTA at pH 7.2) or 1X Optimizer Buffer™ III (Cat. # BKC-06)

PROTOCOL

1. Resuspend 2mg carrier protein in 200µl Coupling Buffer by gentle pipetting.
2. Immediately before use, dissolve 2-10mg sulfhydryl containing peptide in 500µl Coupling Buffer.
3. Mix the peptide and ActiveHOOK™ BSA and allow to react for 2 hours at room temperature.
4. To remove EDTA from the activated protein, purify the conjugate by gel filtration or dialysis. EDTA is an anti-coagulant and should not be injected into laboratory animals.
5. The carrier protein-peptide conjugate is ready for use. The carrier protein-peptide conjugate may be stored at -20°C for later use.

APPENDIX

Ellman's Reagent (DTNB) Assay

1. Make 10mM DTNB stock solution by dissolving 40mg DTNB in 10ml 0.1M Tris-HCl pH 7.5. The stock solution can be stored at 4°C for 3 months. Dilute the stock solution 100 fold with 0.1M Tris-HCl pH 7.5 to make 0.1mM DTNB working solution.
2. Aliquot 950µl of 0.1mM DTNB work solution to each 1.5ml centrifuge tube. Add 50µl test sample and mix by brief vortexing. Set a blank by adding 50µl of 0.1M Tris-HCl pH 7.5 to 950µl of 0.1mM DTNB work solution.
   ***NOTE**: The test sample may need to be diluted before applied to the assay and the dilution factor should be recorded. The 50µl test sample applied to the assay reaction should have a sulfhydryl concentration less than 0.5mM. Concentrations exceeding 0.5mM free sulfhydryl will result in high absorbance values and less accurate estimation of the concentration based on the extinction coefficient.*
3. Incubate 2 minutes at room temperature.
4. Measure the absorbance of the test sample with a spectrophotometer against blank at 412nm.
5. Calculate the concentration of free sulfhydryls in the sample from the molar extinction coefficient of NTB
   ($14.15\ mM^{-1}\ cm^{-1}$) as follow:
   mM free sulfhydryls = Absorbance / (path length x 14.15) x 20 x dilution factor
   Path length is the cuvette path length in centimeter (cm)
   20 is the dilution factor of 50µl sample to 1ml assay volume

Use of SATA to add Sulfhydryls

SATA (N-Succinimidyl S-Acetylthioacetate) (Cat. # BC96) introduce protected sulfhydryls into proteins, peptides and other molecules. It is a NHS esters of S-acetylthioacetic acid.

1. Immediately before reaction, dissolver ~7mg SATA in 0.5ml DMSO to give ~55mM solution.
2. Combine 1ml 2-10mg/ml protein solution in PBS with 10µl 55mM SATA.
3. Incubate at room temperature for 30 minutes
4. Desalt the solution with a desalting column equilibrated with PBS. We recommend G-Biosciences SpinOUT™ GT-600 (Cat. # 786-170).
5. Identify the fraction with the protein using absorbance at 280nm or a suitable assay.
6. Combine 1ml SATA-modified protein with 100µl 0.5M hydroxylamine, 25mM EDTA in PBS.
7. Incubate for 2 hours at room temperature.
8. Desalt as before using PBS supplemented with 10mM EDTA.

Use of Traut's Reagent to add Sulfhydryls

Traut's Reagent (2-Iminothiolane) (Cat. # BC95) is a cyclic thioimidate compound for thiolation of primary amines.

1. Dissolve the protein or peptide in a non-amine buffer at pH8.0. The addition of 2-5mM EDTA will prevent oxidation of generate sulfhydryls into disulfide bridges.
2. Add 2 to 20 fold molar excess of Traut's reagent to the protein solution.
   NOTE: *A 2mg/ml solution of Traut's reagent in water or buffer is a 14mM stock solution.*
3. Incubate the solution for 1 hour at room temperature.
4. Desalt the solution with a desalting column equilibrated with PBS with 2-5mM EDTA. We recommend G-Biosciences SpinOUT™ GT-600 (Cat. # 786-170).

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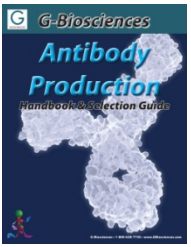

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