Attaching biotin to Bovine Submaxillary Mucin (BSM) and purifying BSM-biotin

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Abstract

This protocol describes how to attach biotin to Bovine Serum Mucin (BSM). The procedure uses a botin-NHS molecule to attach biotin to amines in the mucin proteins.

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Protocol

Step 1.
Calculate how much NHS-Biotin that is needed for biotinylation of the desired amount of BSM (assumed to have M_w 500 000 Da) and the molar ratio of biotin:BSM should be at least 734:1.

Step 2.
Prepare Phosphate Buffered Saline (PBS) containing 137 mM NaCl, 2.7 mM KCl and 10 mM phosphate (should have pH 7-8).

Step 3.
Dissolve BSM in PBS solution and dilute it to a final concentration of 2-10 mg/ml.

Step 4.
Take out the NHS-Biotin reagent and equilibrate it in room temperature before opening the vial.

Step 5.
Dissolve NHS-Biotin in dimethylsulfoxide (DMSO) to a concentration of 10 mM.

Step 6.
Add the pre-calculated volume of biotin reagent to the protein solution and incubate on ice for two hours or at room temperature for 30 minutes.

Step 7.
To remove unbound biotin, use a 15 ml capacity Centricon-10 concentrator with a 50 000 Da cut-off and each time most solution has passed through the filter, add of 20 mM Hepes, 50 mM NaCl buffer. Repeat this at least two times with buffer and two times with milli-Q water.

Step 8.
Transfer the remaining solution in the concentrator to another tube and put in -80°C freezer overnight.

Step 9.
Take out the tube from the -80°C freezer and freeze-dry it for as long as needed.

Warnings

NHS-Biotin reagents are moisture sensitive, so if stored cold, they need to be fully equilibrated to room temperature before opening the vial! Also, the biotin reagent should be prepared right before usage, since it quickly hydrolyses and becomes inactive, i.e. do not prepare stock solutions of the biotin reagent and store them!