Mucin biotinylation - NHS-biotin reagent Version 2

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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 mucin (assumed to have M_w 500 000 Da) and the molar ratio of biotin:mucin should be 1:2000.

ANNOTATIONS
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Use this equation:

ml protein × (mg protein/ml protein) × (mmol protein/mg protein) × (2000 mmol Biotin/mmol protein) = mmol Biotin

Step 2.

Prepare Phosphate Buffered Saline (PBS) with pH 7.4.

Step 3.

Dissolve mucin in PBS to a concentration of 2mg/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 at 4 °C for 4 hours.

Step 7.

To remove unbound biotin, exchange the buffer for water by overnight dialysis.

Step 8.

Transfer the solution to another tube, freeze and freeze-dry.

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!