

Mucin biotinylation - Hydrazide biotin reagent

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Abstract

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Protocol

Step 1.

Prepare these buffers first:

- Oxidation buffer consisting of 0.1 M sodium acetate, pH 5.5
- Coupling buffer consisting of PBS with pH 7.4

Step 2.

Then prepare these solutions:

- 2mg/ml mucin in oxidation buffer
- 20 mM sodium periodate in oxidation buffer

Step 3.

Mix the mucin solution with the sodium periodate solution at a 1:1 (volume) ratio. Incubate at 4 °C for 30 minutes.

Step 4.

Exchange the buffer for coupling buffer by overnight dialysis.

Step 5.

Prepare 50 mM hydrazide biotin reagent in DMSO.

Step 6.

To 9 parts of the mucin solution, add 1 part of the biotin reagent. Mix for 2 hours at RT.

Step 7.

Exchange the buffer for water by overnight dialysis.

Step 8.

Transfer the mucin solution to a tube, freeze and then freeze-dry.