Reaction Protocols for Protein Deglycosylation Mix (P6039)

Overview

Introduction

The quantity of enzyme recommended is sufficient for the deglycosylation of 100 µg of a glycoprotein. Reactions may be scaled-up linearly to accommodate larger amounts of glycoprotein and larger reaction volumes. Optimal incubation times may vary for particular substrates. Typical reaction conditions are as follows:

Protocol

1. **Denaturing Reaction Conditions:**
    1. Dissolve 100 µg of glycoprotein into 18 µl H_2O.
    2. Add 2 µl of 10X Glycoprotein Denaturing Buffer to make a 20 µl total reaction volume.
    3. Denature glycoprotein by heating reaction at 100°C for 10 minutes.
    4. Chill denatured glycoprotein on ice and centrifuge 10 seconds.
    5. To the denatured glycoprotein reaction add 5 µl 10X GlycoBuffer 2, 5 µl 10% NP-40, and 15 µl H_2O.
       PNGase F and O-Glycosidase are inhibited by SDS, therefore it is essential to have NP-40 in the reaction mixture under denaturing conditions. Failure to not include NP-40 into the denaturing protocol may result in loss of activity of some enzymes.
    6. Add 5 µl Deglycosylation Enzyme Cocktail, mix gently.
    7. Incubate reaction at 37°C for 4 hours.
    8. Analyze by method of choice
       Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.
2. **Non-Denaturing Reaction Conditions:**
   When deglycosylating a native glycoprotein it is recommended that an aliquot of the glycoprotein is subjected to the denaturing protocol to provide a positive control for the fully deglycosylated protein. The non-denatured reaction can then be compared to the denatured reaction to determine the extent of reaction completion.
    1. Dissolve 100 µg of glycoprotein into 40 µl H_2O.
    2. To the native glycoprotein add 5 µl 10X GlycoBuffer 2.
    3. Add 5 µl Deglycosylation Enzyme Cocktail, mix gently.
    4. Incubate reaction at 37°C for 4 hours.
       Note: To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.
    5. Analyze by method of choice.
       Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.