Deoxyribonucleic acid (DNA) methylation is widespread in eukaryotic organisms and is known to have a role in the regulation of gene expression. Methylation of cytosine residues in DNA, typically at the 5-position, is catalyzed by DNA methyltransferases (DNMTs), which transfer a methyl group from S-adenosylmethionine (SAM) to the DNA substrate. This process is essential for the maintenance of DNA methylation balances, which are critical for the stability of epigenetic profiles and the proper functioning of the genome. In addition to DNA methylation, other modifications such as histone modifications and non-coding RNA transcripts play a crucial role in the regulation of gene expression. These modifications can influence gene expression through different mechanisms, including the accessibility of transcription factors to the DNA, histone modifications that affect chromatin structure, and the recruitment of co-activators or co-repressors to the transcriptional machinery. Understanding these epigenetic mechanisms is crucial for the development of therapeutic strategies targeting gene expression regulation in various disease states.
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.*

**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 H₂O.
2. To the native glycoprotein add 5 µl 10X G7 Reaction Buffer.
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.*

**Storage**

It is recommended to store this kit at 4°C. All components of the kit will be stable for at least one year if stored correctly.

**Notes:** Deglycosylation Mix is not recommended for use on Mucin-like substrates.

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**References**


U.S. Patent No. 6,358,724 and 5,770,405.