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ProductInformation

Ribonuclease B
Glycoprotein Standard, Proteomics Grade
from bovine pancreas

Product Number **R1153**
Storage Temperature –20 °C

TECHNICAL BULLETIN

EC 3.1.27.5
Synonym: RNase B

Product Description

Glycosylation is one of the most common post-translational modifications of proteins in eukaryotic cells. Glycoproteins are involved in a wide range of biological functions such as receptor binding, cell signaling, immune recognition, inflammation, and pathogenicity. Mammalian glycoproteins contain three major types of oligosaccharides (glycans): N-linked, O-linked, and glycosylphophatidylinositol (GPI) lipid anchors. N-Linked glycans are linked to the protein backbone via an amide bond to asparagine residues in an Asn-Xaa-Ser/Thr motif, where Xaa can be any amino acid, except proline.

Bovine pancreatic Ribonuclease B (RNase B) is a glycoprotein that contains only N-linked glycans. It is a globular protein composed of a single domain that occurs naturally as a lesser component in a mixture along with Ribonuclease A (RNase A), which is the non-glycosylated core form. Both forms have an identical amino acid sequence and show the same basic catalytic activity.[1] RNase A has a molecular weight of 13.7 kDa. RNase B contains a single glycosylation site at Asn^{34}. The glycan varies from five to nine mannose residues attached to the chitobiose core ($Man_5GlcNAc_2$, $Man_6GlcNAc_2$, $Man_7GlcNAc_2$, $Man_8GlcNAc_2$, and $Man_9GlcNAc_2$).[2, 3] Due to the heterogeneity in the glycosylation at Asn^{34}, RNase B exists as five glycosylated variants, with a molecular weight of ~15 kDa. The presence of these oligomannose sugars changes some of the properties of RNase B. It has an increased resistance to proteolysis, but a decreased rate of reaction with double stranded RNA compared to RNase A.[3]

RNase B is a preferred substrate with PNGase F for demonstration of N-linked deglycosylation using SDS-PAGE or MALDI-MS. The activity of PNGase F is also routinely assayed using RNase B by monitoring the pronounced mobility shift in 12% gels after deglycosylation.[4, 5] RNase B can also be used as a source of N-glycans following enzymatic digestion and subsequent purification. For these applications it is preferable to use an RNase B that is free of contaminating RNase A. The RNase B Glycoprotein Standard has been highly purified to remove contaminating RNase A and is an excellent glycoprotein standard for the above applications.

Component

The Ribonuclease B Glycoprotein Standard is provided as 0.5 mg of lyophilized protein in a screw cap vial. The user may reconstitute the contents in the vial to the required protein concentration.

Reagents Required But Not Provided

(Product Codes have been supplied)
Proteomics Grade PNGase F (50 units),
Product Code P7367
2-Mercaptoethanol, Product Code M6250
Octyl β-D-glucopyranoside, Product Code O9882
Ammonium bicarbonate, Product Code A6141

Precautions and Disclaimer

This product is for laboratory use only, not for drug, household, or other uses. Consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

It is recommended to read the entire technical bulletin prior to starting the procedure.

Preparation Instructions
It is recommended to use ultrapure (18 MΩ×cm or equivalent) water when preparing the reagents.

- Reaction Buffer – Weigh out 160 mg of ammonium bicarbonate and dissolve in 100 ml of ultrapure water. Store at 2–8 °C and use within two weeks of preparation.
  Note: 50 mM sodium phosphate, pH 7.5, may be used as an alternative reaction buffer, but the sample may require desalting prior to mass spectrometric analysis.
- RNase B Standard Solution – Centrifuge vial briefly to collect solid at the bottom. Add 0.45 ml of the Reaction Buffer, agitate gently to dissolve the solid, and centrifuge briefly to obtain a 1.1 mg/ml solution. Store at 2–8 °C and use within four weeks of preparation.
- PNGase F Solution – Centrifuge the vial (50 units) briefly to collect solid at the bottom. Add 0.1 ml of ultrapure water, agitate gently, centrifuge briefly, and store on ice for 5 minutes. Mix the contents gently once more and centrifuge briefly. The concentration is 500 units/ml. Store at 2–8 °C and use within one week of preparation.
- Denaturant Solution – Weigh out 100 mg of octyl β-D-glucopyranoside and add to 4 ml of water. Mix to dissolve and then add 35 µl of 2-mercapto-ethanol followed by 0.965 ml of water to obtain a Denaturant Solution containing 2% octyl β-D-gluco-pyranoside and 0.1 M 2-mercaptoethanol. Store at 2-8 °C and use within four weeks of preparation.

Storage/Stability
This product is stable for at least one year if stored unopened at –20 °C. A reconstituted solution can be stored at 2–8 °C for up to 4 weeks.

Procedure
This procedure is a convenient method for using the RNase B standard to demonstrate N-deglycosylation by PNGase F. It is compatible with subsequent MALDI-TOF mass spectrometric analysis without interference from the reaction components.

The quantity of PNGase F enzyme recommended in the following procedure is sufficient to deglycosylate 50 µg of RNase B in one hour with incubation at 37 °C.

1. Add 90 µl of the 1.1 mg/ml RNase B Standard Solution to a small Eppendorf® tube.

2. Add 5 µl of Denaturant Solution containing 2% octyl β-D-glucopyranoside and 0.1 M 2-mercaptoethanol. Mix and centrifuge briefly.

3. Incubate at 100 °C for 10 minutes, making sure the cap is firmly closed to prevent evaporation.

4. Allow the solution to cool to room temperature and centrifuge briefly.

5. Add a further 5 µl of Reaction Buffer, mix, and centrifuge. This makes a 1 mg/ml solution of RNase B.

6. Split the solution by transferring 50 µl to a separate tube. Label one tube as the control and the other as the test.

7. To the tube labeled test, add 4 µl of the PNGase F Solution (equivalent to 2.0 units of enzyme). To the control sample, add 4 µl of water.
   Note: The amount of enzyme may be varied for other glycoproteins, depending upon the nature of the glycosylation.

8. Mix and centrifuge briefly.

9. Incubate at 37 °C for 1 hour.
   Note: The incubation time may also be varied for partial or complete deglycosylation.

10. Stop the reaction by heating at 100 °C for 10 minutes.

11. Allow the solution to cool and centrifuge briefly.

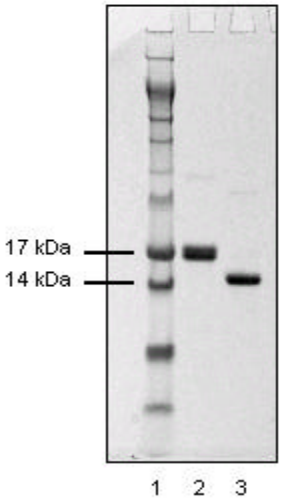
12. Analyze an aliquot of the reaction mixture by SDS-PAGE to assess deglycosylation.
    Note: The reaction mixture may also be lyophilized for subsequent MS analysis of the deglycosylated protein or of the released oligosaccharides (after appropriate treatment).

The procedure provided above has been optimized for use with the RNase B Glycoprotein Standard. When using other substrates the reaction conditions may require optimization of incubation time and PNGase F concentration for complete deglycosylation.

Results

One of the simplest methods to assess the extent of deglycosylation is by mobility shift on SDS-PAGE gels. Figure 1 shows the deglycosylation of RNase B using 2.0 units of PNGase F Solution. The control RNase B sample (lane 2) has an apparent M_r of 17 kDa compared to an apparent M_r of approximately 15 kDa for the test RNase B sample digested with PNGase F (lane 3). The control band is completely absent from lane 3 indicating complete deglycosylation. The ability to detect mobility shifts when the N-linked oligosaccharides are removed with PNGase F will depend on the molecular weight of the protein and the relative mass contribution by the oligosaccharide.

Figure 1.
In-Solution Deglycosylation of RNase B by PNGase F Enzyme



Analysis of the deglycosylation of RNase B on 12% SDS-PAGE gel. Lane 1 is a set of molecular weight standards. Lane 2 is the control sample of denatured RNase B, while lane 3 is a test sample treated with 2.0 units of PNGase F.

Related Products	Product Code
GlycoProfile™ I Enzymatic In-Gel N-Deglycosylation Kit	PP0200
GlycoProfile II Enzymatic In-Solution N-Deglycosylation Kit	PP0201
Trypsin Profile IGD Kit	PP0100
ProteoMass™ MALDI-MS Calibration Kits	
Peptide and Protein	MSCAL1
Peptide	MSCAL2
Protein	MSCAL3
Trypsin, Proteomics Grade	T6567
PNGase F, Proteomics Grade	P7367
Invertase, Glycoprotein Standard, Proteomics Grade	I0408

References

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