

Certificate of Analysis

Protein Deglycosylation Mix:

Cat.# V4931

| Part# | Component | Size |
|-------|-------------------------------------|-------|
| V493A | Protein Deglycosylation Mix | 100µl |
| V492A | 10X Denaturing Solution | 1ml |
| V494A | 10X Deglycosylation Reaction Buffer | 1ml |
| V495A | 10% NP-40 | 1ml |
| V496A | Fetuin | 50µl |

Description: Protein Deglycosylation Mix (Part# V493A) is a mixture of five protein deglycosidases (PNGase F, O-Glycosidase, Neuraminidase, β 1-4 Galactosidase, β -N-Acetylglucosaminidase) capable of removing glycans from both O-linked and N-linked glycosylation sites. Fetuin (Part# V496A) is provided as a deglycosylation substrate control.

Molecular Weights: PNGase F has an apparent molecular weight of approximately 36kDa. O-Glycosidase has an apparent molecular weight of approximately 147kDa. Neuraminidase has an apparent molecular weight of approximately 43kDa. β 1-4 Galactosidase has an apparent molecular weight of approximately 94kDa. β -N-Acetylglucosaminidase has an apparent molecular weight of approximately 71kDa. Fetuin has a molecular weight of 50kDa or approximately 42.7kDa when deglycosylated.

Storage Conditions: Store at 2°C to 10°C. For long-term storage, store 10X Denaturing Solution and Fetuin at -30°C to -10°C.

Physical Form: Protein Deglycosylation Mix (Part# V493A) is supplied in 20mM Tris-HCl (pH 7.5 at 25°C), 50mM NaCl and 5mM EDTA.

Denaturing Solution: 10X Denaturing Solution (Part# V492A) is composed of 5% SDS, 0.4M DTT.

Reaction Buffer: 10X Deglycosylation Reaction Buffer (Part# V494A) is composed of 0.5M sodium phosphate (1X pH 7.5 at 25°C).

Usage Note: O-Glycosidase and PNGase F are both inhibited in the presence of SDS. NP-40 is needed for deglycosylation of glycoproteins under denaturing conditions with SDS.

Part# 9PIV493

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Promega

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Signed by:

J. Stevens, Quality Assurance

1. SDS-PAGE Analysis of Samples Treated with the Protein Deglycosylation Mix

Glycoprotein Denaturation:

1. Add 1–50µg of the target glycoprotein to an appropriate amount of water (or buffer (pH 7.5)) to a final volume of 18µl.
Note: Fetuin (Part# V496A) may be used as a positive control. Fetuin is supplied at 10mg/ml; 5µl of Fetuin is recommended in a total volume of 18µl.
2. Add 2µl of 10X denaturing solution (0.5% SDS, 40mM DTT, 1X concentration) to give a denaturing reaction volume of 20µl.
3. Heat the sample for 10 minutes at 95°C.
4. Place the samples on ice for 5 minutes.

Deglycosylation Reaction:

5. Add 5µl of 10X Deglycosylation Reaction Buffer, 5µl of 10% NP-40 and 15µl of water to the denatured glycoprotein.
6. Add 5µl of Protein Deglycosylation Mix to the tube to give a final reaction volume of 50µl.
7. Vortex the sample gently.
8. Centrifuge the sample to collect sample at the bottom of the tube.
9. Incubate for 4–18 hours at 37°C.
10. Analyze the products using SDS-PAGE.

Note: PNGase F and Endo- α -N-Acetyl-galactosaminidase (O-Glycosidase) are inhibited by SDS. Include NP-40 in the reaction to prevent loss of activity of these enzymes.

2. Mass Spectrometry Analysis

Note: The denaturing step is omitted.

1. Combine 1–50µg of the target glycoprotein to an appropriate amount of water (or buffer) to give a final volume of 40µl.
Note: Fetuin (Part# V496A) may be used as a positive control. Add 5µl of Fetuin to 35µl of water.
2. Add 5µl of 10X Deglycosylation Reaction Buffer to the tube to give a final reaction volume of 45µl.
3. Add 5µl of Protein Deglycosylation Mix to the tube.
4. Vortex the sample gently.
5. Centrifuge the sample to collect sample at the bottom of the tube.
6. Incubate the reaction for 4–18 hours at 37°C.
7. At this point the samples are ready for MS analysis using either solution- or gel-based digestion protocols (1). To desalt the sample prior to mass spectrometric analysis, see the ZipTip® protocol given in the *Trypsin Gold, Mass Spectrometry Grade, Technical Bulletin #TB309*.

3. Related Products

| Product | Size | Conc. | Cat. # |
|---|------------------|---------|--------|
| Asp-N, Sequencing Grade | 2µg | | V1621 |
| Arg-C, Sequencing Grade | 10µg | | V1881 |
| Chymotrypsin, Sequencing Grade | 25µg | | V1061 |
| | 100µg (4 × 25µg) | | V1062 |
| Elastase | 5mg | | V1891 |
| Endo H | 10,000u | 500u/µl | V4871 |
| | 50,000u | 500u/µl | V4875 |
| Endoproteinase Lys-C, Sequencing Grade | 5µg | | V1071 |
| Fetuin | 500µg | 10mg/ml | V4961 |
| Glu-C, Sequencing Grade | 50µg (5 × 10µg) | | V1651 |
| Immobilized Trypsin | 2ml | | V9012 |
| | 4ml (2 × 2ml) | | V9013 |
| Pepsin | 250mg | | V1959 |
| PNGase F | 500u | 10u/µl | V4831 |
| ProteaseMAX™ Surfactant, Trypsin Enhancer | 1mg | | V2071 |
| | 5 × 1mg | | V2072 |
| rLys-C, Mass Spec Grade | 15µg | | V1671 |
| Sequencing Grade Modified Trypsin | 100µg (5 × 20µg) | | V5111 |
| Sequencing Grade Modified Trypsin, Frozen | 100µg (5 × 20µg) | | V5113 |
| Thermolysin | 25mg | | V4001 |
| Trypsin Gold, Mass Spectrometry Grade | 100µg | | V5280 |
| Trypsin/Lys-C Mix, Mass Spec Grade | 20µg | | V5071 |
| | 100µg | | V5072 |
| | 100µg (5 × 20µg) | | V5073 |

4. Reference

1. *Trypsin Gold, Mass Spectrometry Grade*, Technical Bulletin #TB309, Promega Corporation.