

Non-denaturing deglycosylation of IgG:

MALDI-TOF compatible application.

1. IgG desalting using an Amicon Ultra-0.5 mL Centrifugal Filter device MWCO 30 kDa:
Add your IgG sample to the filter device. If volume is less than 400 μ L, add ultra pure water up to 400 μ L and spin the sample for 5 minutes at 9,000x g at room temperature. Discard the flow-through. Repeat for a total of three times.
Desalting of antibody is recommended as the presence of salts and detergents could affect the MALDI-TOF-MS acquisition
2. Add 1 μ L of reconstituted *CarboClip* enzyme for each 50-200 μ g of IgG to deglycosylate. Mix the sample by flicking (do not vortex or pipette up and down). Incubate the mixture at 60°C for 1 hour with gentle shaking.

For efficient deglycosylation of the Fab region IgG denaturation could be necessary.

Denaturing deglycosylation of glycoproteins:

After conventional glycoprotein denaturation⁽¹⁾ add 1-3 μ L of reconstituted *CarboClip*, for each 0.5-2 mg of glycoprotein to deglycosylate. Incubate reaction at 37°C for 1 hour.

CarboClip is inhibited by SDS, therefore it is essential to have NP-40 in the reaction mixture under denaturing conditions. Besides, optimal incubation times may vary depending on the glycoprotein.

(1) *Nature Protocols 2, - 1585 - 1602 (2007)*

CarboClip

2500 IUB mU

+34 943 000 999

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INSTRUCTIONS

Instruction for CarboClip (2500 IUB mU).

Upon arrival store lyophilized *CarboClip* at -20 °C.

Introduction

Peptide -N-Glycosidase F, also known as PNGase F, is an amidase that cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from N-linked glycoproteins.

PNGase F is not able to cleave N-linked glycans from glycoproteins when the innermost GlcNAc residue is linked to an α 1-3 Fucose residue.

After enzyme purification *CarboClip* is lyophilized and the activity of the reconstituted enzyme is comparable to that of freshly purified protein.

CarboClip is provided glycerol-free as a lyophilized powder containing Tris-HCl, NaCl, and Na₂EDTA.

Reconstitution of lyophilized *CarboClip*

1. Centrifuge the vial before opening at 1,000 x g for 1 minute.
(To dislodge any lyophilized material that may be dispersed on the wall or cap of the vial).
2. Add 200 μ l of ultraPure water to the tube.
- *If higher concentration per μ l is needed reduce the water volume.*
- *UltraPure water should be at room temperature for an optimal reconstitution.*
3. Re-cap the vial and invert gently by hand.
(Do not mix by vortexing or by pipetting the material up and down).
4. Allow the vial to sit at room temperature with gentle agitation for 10 minutes.
5. Centrifuge the reconstituted enzyme at 1,000 x g for 1 minute.
6. Once reconstituted, keep *CarboClip* at 4 °C.

Storage

Reconstituted *CarboClip* should be stored at 4 °C, and is stable for 2 months without significant loss of activity.

At room temperature lyophilized *CarboClip* has been tested to be stable for 10 days without significant loss of activity.

Stored at -20 °C or - 80 °C *CarboClip* is stable >1 year without significant loss of activity.