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 µL, add ultra pure water up to 400 µL 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 µL of reconstituted *CarboClip* enzyme for each 50-200 µg 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 µl of reconstituted CarboClip, for each 0.5-2 mg of glycoprotein to deglycosylate. Incubate reaction at 37°C for 1 hour.
A complete protocol for deglycosylation after denaturation can be found in the Lot analysis test page attached with this shipment.
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

500 IUB mU

+34 943 000 999

https://aspariaglycomics.com
Parque Científico y Tecnológico de San Sebastián
Paseo de Mikeletegi, 83
20009 San Sebastián
SPAIN

INSTRUCTIONS

Instruction for CarboClip (500 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 α1-3 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 Na_2EDTA.

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 50 µl of ultraPure water to the tube.
   (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.