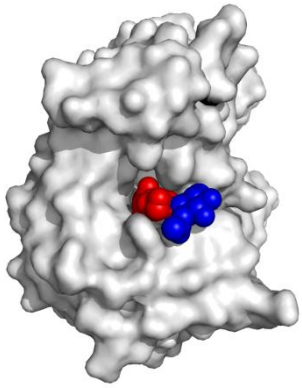
CarboClip



CarboClip is a 37.2 kDa recombinant peptide-N-Glycosidase F (PNGase F), from *Elizabethkingia sp*, overexpressed in *E. coli* that cleaves between the GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from N-linked glycoproteins.
The absence of glycerol makes this enzyme compatible with Mass Spectrometry applications.

CarboClip is provided glycerol-free as a lyophilized powder containing Tris-HCl, $NaCl_2$, and Na_2EDTA. Stored at -80 °C it has a shelf life of > 1year, In lyophilized form it can be can be shipped at room temperature. For larger volumes (>100 vials, 10.000Units) we offer customized enzyme aliquotation/packaging.

Unit definition

One CarboClip unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 minute at 37°C in a total reaction volume of 10 µl.

We strive to offer you the best price of the market. Minimum order 5 vials (500 IUB milliunits).

Notes for Use:

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 30 µl of the 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

At room temperature lyophilized CarboClip® has been tested to be stable for 10 days without significant loss of activity.
Reconstituted *CarboClip®* should be stored at 4 °C, and is stable for 2 months without significant loss of activity.
Stored at - 20 °C *CarboClip®* is stable >1 year without significant loss of activity.
Stored at - 80 °C *CarboClip®* is stable >1 year without significant loss of activity.

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 3-6 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 3-6 µl of reconstituted CarboClip, for each 50-200 µg 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 of the glycoprotein.

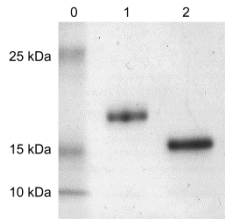


FIG.1. Shown is a 12% SDS-PAGE showing the migration pattern of denaturated RNAse B before (lane 1) and after the addition of CarboClip (lane 2). Theoretical Molecular Weight of glycosylated/deglycosylated proteins are 17 kDa and 15 kDa, respectively.

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