

CarboClip lot AD0CB0AG analysis

Activity test

After reconstitution in 600 µL of UltraPure H₂O, 0.01 µl of CarboClip can deglycosylate > 95% of ribonuclease B (10 µg) in less than 20 minutes. Activity is calculated on a 12 % SDS-PAGE and percent of deglycosylation using densitometry.

	<u>Estimated activity/vial</u>	<u>Real Tested activity/vial</u>
CarboClip lot AD0CB0AG	2,500 IUB mU	~ 3,692 IUB milliunits

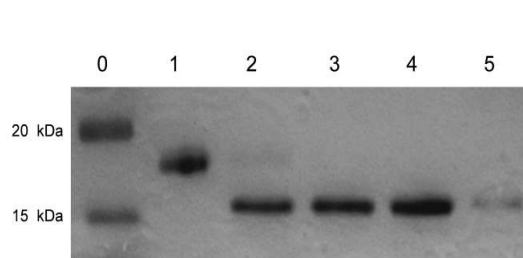
Method used for the activity test

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 600 µl of the ultraPure water (milliQ, NanoPure, or similar).
UltraPure water should be at room temperature for an optimal reconstitution
3. After adding ultraPure water, 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 5 minutes.
5. Centrifuge the reconstituted enzyme at 1,000 x g for 1 minute

Test is done using the enzyme diluted 1:100 in PNGase F buffer (20 mM Tris-HCl, 50 mM NaCl, 5 mM Na₂EDTA, pH 7.5). For the dilution, first the enzyme is diluted 1:10, and from this 1:10.

1. In a 10 µl reaction, added 2 µl of RNase B (at 5µg/µL), 1 µl of Denaturing Buffer (5% SDS, 400 mM DTT) and 5 µl of NanoPure H₂O.
2. Incubated at 100°C for 10 minutes.
3. Added 1 µl of 10X reaction buffer 500 mM Sodium Phosphate (pH 7.5) and 1 µl of 10% NP-40.
4. Added 1 µl of Carboclip (diluted 1:100).
5. Incubated at 37°C and taken 2 µL samples after exactly 10 min, 30 min and 1 hour.
6. Before loading ,samples are placed at 100 °C for 10 min.
7. Deglycosylated/Glycosylated RNase B are visualized using a 12% precast BIO-RAD SDS-PAGE gel. Ran at 200 volts (0.03 Amp) for 40 min. Stained with Coomassie blue.



*RNase B Theoretical Molecular Weight:
Glycosylated 17 kDa, Deglycosylated 15 kDa.*

Deglycosylated/Glycosylated RNase B are visualized using a 12% precast BIO-RAD SDS-PAGE gel. Ran at 200 volts (0.03 Amp) for 30 min. Stained with Coomassie blue. Dual Color Standards#1610374 (lane 0), No CarboClip (lane 1), 3 ng of CarboClip after 10 min (lane 2), 3 ng of CarboClip after 30 min (lane 3), 3 ng of CarboClip after 1 hour (lane 4), CarboClip Positive control (lane 5)