Asparia Glycomics

**IgG deglycosylation analysis using MALDI-TOF**

1. Add 2 µl of CarboClip (PNGase F) to 100 µl of IgG (10 µg/µl) diluted in nanoPure $H_2O$.
2. Incubate at 60 ºC for 2h, agitating at 300 rpm.

*Next steps are used to remove the remaining buffer from the PNGase F that interferes with the MALDI.*

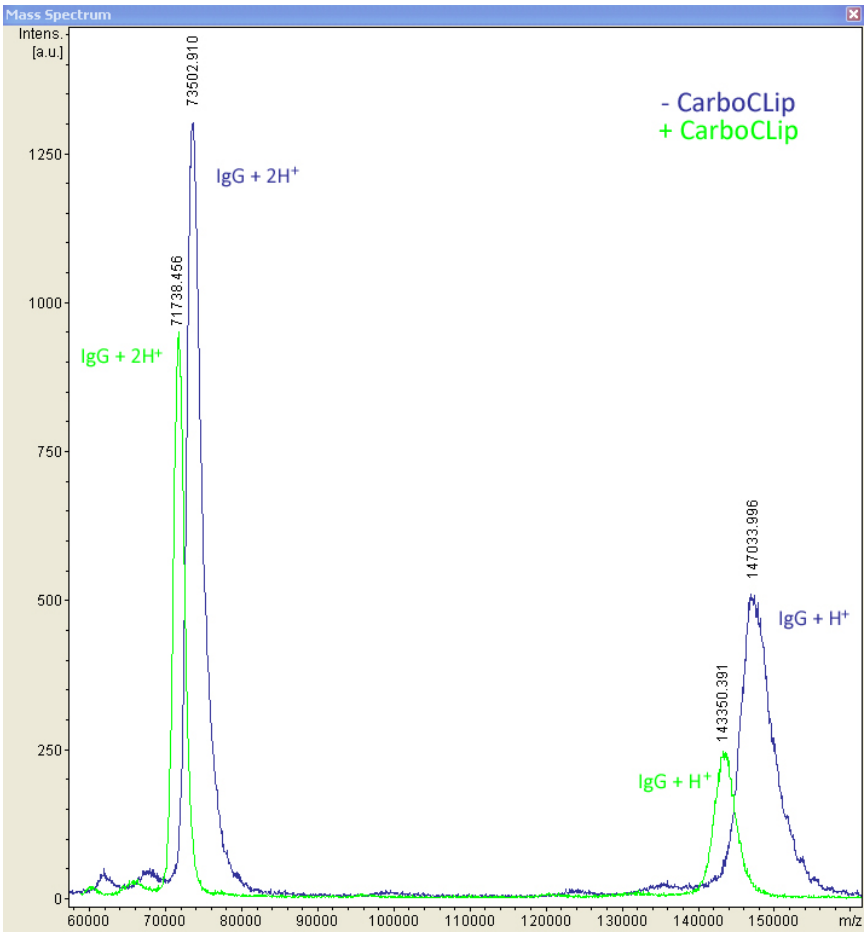
3. Add the 102 µl to a 30 kDa amicon device and add 350 µl more of nanoPure $H_2O$. Centrifuge at 10,000 x g for 5 min at RT. Remove filtered sample.
4. Add again another 400 µl more of nanoPure $H_2O$. Centrif. at 10,000 x g for 5 min at RT. Remove filtered sample (repeat this step twice).
5. Invert the amicon device and recover sample by centrifugation at 1,000 x g for 3 min at RT. Recover concentrated IgG.

*MALDI-TOF sample preparation and analysis*

6. Add 1 µl of concentrated IgG to the polished steel MALDI-TOF plate, and add 1 µl of SuperDHB (diluted in ACN at 20 µg/µl). Dry under vacuum or at RT.

*Flex control-ultraflex tof-tof software*

7. Method: *LP_30-210_KDa.par.*
8. Set laser Attenuator *"3_medium*".
9. Mass range 68,000 – 170,000.
10. Laser intensity 90-100%




Accumulated Spectra of IgG before (Blue) and after adding CarboClip (Green) is shown.
Single charged and double charged IgG are shown

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