PolyMAC-Ti enrichment - high selectivity

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1) Preparation of samples obtained from protein digest

Prepare a peptide sample with low salt concentration. Desalting step is recommended if a sample contains high concentration of salt or detergent (e.g. after digestion). Dry the sample completely in a low-binding microfuge tube using Speedvac. Store at -80 if not used for immediate enrichment.

2) Wash and equilibration of capture gel

Mix the **Capture gel (Affi-Gel Hydrazide gel)** slurry well and carefully transfer 50 µl (cut off the tip for efficient transfer) of the **Capture gel** slurry to a Boca Scientific spin column and spin down at 5,000 rpm for 30 seconds to remove solution (remove bottom cap before each spindown). Wash the gel twice with 200 µl of **water** by adding water to the spin column with beads, briefly vortexing the beads and spin-down at 5,000 rpm for 30 seconds.

Note: dry the bottom cap with a kimwhipe and place it back at the bottom of the spin column before adding each solution between every washing step).

3) Binding

Add 100 µl of **Loading buffer** (100mM glycolic acid, 1% TFA, 50% ACN) into the dried peptide sample in a non-stick microfuge tube and resuspend well. Add 10 µl of **PolyMAC reagent** and vortex 10 seconds. Vigorously shake the solution for 5 minutes at ~1,200 rpm. Add 200 µl of **Capture buffer** (300mM HEPES, pH 7.7) to increase the pH above 6.3 and vortex briefly. If pH is still below 6.3 (check with pH paper), add more **Capture buffer.**

4) PolyMAC capture

Pipette the mixture up-and-down a few times and transfer the whole solution to the washed capture gel in the spin column and shake vigorously for 10 minutes at ~1,200 rpm. Spin down the gel at 5,000 rpm for 30 seconds to remove flowthrough.

5) Capture gel wash

Incubate the gel once with 200 µl of **Loading buffer** for 5minutes at ~1,200 rpm and spin down the beads at 5,000 rpm for 30 seconds. Then incubated the beads twice (5 minutes each time) at ~1,200 rpm with **Washing buffer** (100mM acetic acid, 1%TFA, 80% acetonitrile) and spin down the beads at 5,000 rpm for 30 seconds. Finally, incubate the beads once with 200 µl of **water** for 5 minutes at ~1,200 rpm and spin down the beads at 5,000 rpm for 30 seconds.

6) Sample elution

Incubate (shake) the gel twice with 100 µl of **Elution buffer** (400mM ammonium hydroxide) for 5 minutes each time at ~1,200 rpm and collect the eluents into the same low-binding tube by spinning down at 5,000 rpm for 30 seconds.

7) Sample preparation for MS

Dry the eluents completely in a vacuum centrifuge and resuspend in10 µL of **0.5% formic acid**. Centrifuge the resuspended samples at max speed (e.g. 13,000 rpm) for 10 min and carefully transfer 9 µL of the solution into an LC/MS vial (**Very important**: be careful not to disturb the white precipitate that forms at the bottom).