Protocol

PolyMAC-Ti phosphopeptide enrichment using spin-tips

Due to low stoichiometry of phosphorylation, phosphopeptide enrichment is a critical step for successful phosphoproteomics experiments. PolyMAC-Ti offers an efficient and greatly improved method to achieve more complete phosphopeptide enrichment. This highly selective enrichment procedure can be used with majority of the complex samples because it provides optimal specificity and recovery.

PolyMAC enrichment procedure

1) Preparation of samples obtained from protein digest

Prepare and completely dry a peptide sample with low salt concentration in a non-stick microtube. Desalting step is recommended if a sample contains salt or detergent (e.g. after digestion).

2) PolyMAC binding to phosphopeptides

Completely resuspend the dry sample with 200 µl* of **Loading buffer**. Briefly vortex (~10-20sec) the **PolyMAC** beads to uniformly resuspend the beads and transfer 50 µl* of the **PolyMAC** beads to a new non-stick tube (Note: the beads will pull to the bottom very quickly, so it is best to vortex the beads before each transfer). Spin down the beads 2-3 secs and remove the storage solution. Add the resuspended sample to the beads and shake vigorously for 20 minutes at >1,200 rpm. **Important**: Make sure that the microtubes are secured in the shaker and the beads are uniformly moving around in the solution.

3) Removal of flowthrough

Put a tip with the frit into a microfuge tube with the provided centrifuge adaptor (or use an alternative way to secure the tip in the tube). After incubation, add the sample with the PolyMAC beads to the tip and centrifuge at 100 x g for 1min. The solution (flowthrough) should now be in the tube and out of the tip.

4) Capture beads wash

Add 200 µl of **Loading buffer** to the tip and centrifuge once for 2min at 20 x g and once for 1min at 100 x g.
Add 200 µl of **Washing buffer 1** to the tip and centrifuge once for 2min at 20 x g and once for 1min at 100 x g.
Add 200 µl of **Washing buffer 2** to the tip and centrifuge once for 2min at 20 x g and once for 1min at 100 x g

5) Sample elution

Put the tip with beads into a fresh tube to collect the eluted phosphopeptides. Add 50 µl of **Elution buffer** to the tip and centrifuge once for 2min at 20 x g. Add another 50 µl of **Elution buffer** to the tip and centrifuge once for 2min at 20 x g. Centrifuge one final time for 1min at 100 x g. If any solution remains in the tip, push it out into the collection tube with a pipette.

6) Sample preparation for MS

Dry the eluents completely in a vacuum centrifuge (can be dried directly in LC-MS vials; for faster drying, it helps to freeze the eluted samples first). The sample is ready for LC-MS analysis (optimal resuspension solution is **0.25% formic acid in 3% acetonitrile**).

*Capacity note: 50 µl of PolyMAC is designed for efficient enrichment of standard samples (e.g. 100 µg or less of peptide sample). For larger peptide amounts the PolyMAC beads volume and loading buffer volume should be increased accordingly. The optimal ratio should be tested for each sample type and could change depending on the amount of phosphorylation in the sample.

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Store the PolyMAC beads at 4°C
Store the buffers at room temperature