Protocol

One-step phosphopeptide enrichment by PolyMAC-Ti using magnetic beads

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 under homogeneous conditions. 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 first with 20 µl of ultrapure water. Add 200 µl* of **Loading buffer** to the sample and vortex. Briefly vortex (~20-30sec) the **PolyMAC/Magnetic Capture beads** mixture to uniformly resuspend the beads, add 50 µl* of the **PolyMAC/Magnetic Capture beads** to the sample* and shake vigorously for 10 minutes at ~700-1000 rpm. **Important**: Make sure that the microtubes are secured in the shaker and are not loose during each incubation step; this will ensure that the capture beads are uniformly moving around in the solutions.

Remove the solvent by briefly spinning down the beads (for 2-3sec) and using the magnetic separator rack: place the microtube in a magnetic separator rack, wait for 30sec until the beads are pulled to the side, turn the microtube 90º to pull the beads to the side again and carefully discard the solution with a pipette.

4) Capture beads wash

Incubate the beads with 200 µl of **Washing buffer 1** for 5 minutes at ~700-1000 rpm and remove the solvent using the magnetic separator rack. Wash again with **Washing buffer 2** for 5min and remove the solvent.

5) Sample elution

Incubate the beads twice with 50 µl of **Elution buffer** for 5 minutes each time at ~700-1000 rpm and collect 45-48 µl each time into the same low-binding microtube by using the magnetic separation rack (this solution contains the phosphopeptides). The remaining 5 µl of eluent might contain some bead contaminants and should be left behind with the beads.

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/beads mixture 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.