Cube Biotech

Phosphopeptide enrichment using PureCube magnetic beads (25 % suspension)

Ti-NTA cat no 31501-Ti; 31505-Ti; 31525-Ti
Zr-NTA cat no 31501-Zr; 31505-Zr; 31525-Zr
Fe-NTA cat no 31501-Fe; 31505-Fe; 31525-Fe

Comments

- The protocol has been used for 250 µg HELA cell digest and 2.5 µl beads (=10 µl of the 25% bead suspension). Adjust the volumes of wash and binding buffers for higher bead/protein amounts
- 0 – 5 % GA is added to the binding buffer to enhance specificity. The optimal amount depends on-samples and can be optimized

Abbreviations

- ACN acetonitrile, TFA trifluoroacetic acid, FA formic acid, GA glycolic acid

Reagents

- Tryptic protein digest, C18 desalted, dry in 1.5 ml tubes
- PureCube magnetic beads (Ti-NTA or Zr-NTA or Fe-NTA) , 25 %
- Binding buffer: 80% ACN 5 % TFA, 0.2% GA
- Wash buffer 1: 80 % ACN, 1 % TFA
- Wash buffer 2: 10 % ACN, 0.2 % TFA
- Elution buffer: 1 % ammonium hydroxide

Procedure

- use 10:1 (w/w) bead to protein ratio
- calculate the required amount of beads for all samples + 1. Wash three times in 10 x volume of binding buffer and adjust the final concentration to 2.5 % in binding buffer
- add 250 µl binding buffer to dried protein digest and incubate for 5 min on a thermoshaker to resuspend the peptides (250 rpm, 25°C)

- add the required amount of beads and incubate for 20 min on a thermoshaker (250 rpm, 25°C)
- place tube on a magnetic rack until all beads are trapped (10 - 30 sec) and remove supernatant
- add 250 µl wash buffer 1 , incubate for 2 min on a thermoshaker (250 rpm, 25°)
- place tube on a magnetic rack until all beads are trapped (10 - 30 sec) and remove supernatant
- add 250 µl wash buffer 1 , incubate for 2 min on a thermoshaker (250 rpm, 25°C
- place tube on a magnetic rack until all beads are trapped (10 - 30 sec) and remove supernatant
- add 250 µl wash buffer 2, incubate for 4 min on a thermoshaker (250 rpm, 25°C)
- place tube on a magnetic rack until all beads are trapped (10 - 30 sec) and remove supernatant
- add 80 µl elution buffer and incubate for 30 min on a thermoshaker (250 rpm, 25°C)
- place tube on a magnetic rack until all beads are trapped (10 - 30 sec) and transfer supernatant to a new tube
- add 20 µl 10 % FA to supernatant and dry in a vacuum centrifuge