

Phosphopeptide enrichment with magnetic immobilized metal-ion (Fe³⁺) chromatography

Materials

- ✓ Magnetic Ni-NTA-agarose beads were obtained from Qiagen (Valencia, CA), N#36111
- ✓ 100 mM EDTA, pH 8.0
- ✓ 10 mM aqueous metal-ion solution
- ✓ 1:1:1 acetonitrile/methanol/0.01% acetic acid
- ✓ Wash/resuspending buffer - 80% acetonitrile with 0.1% TFA
- ✓ Elution Buffer- 1:1 acetonitrile/1:20 ammonia/water

Bead preparation

Ni-NTA beads were supplied as a 5% bead suspension. A 50 μL aliquot of the 5% suspension of metal-ion activated NTA was used per 100 μg of peptides.

1. Appropriate aliquot of Ni-NTA beads was washed 3 \times with nano-pure water (800.0 μL of water per 1.0 mL of bead suspension).
2. Beads were treated with 100 mM EDTA, pH 8.0 (800.0 μL of 100 mM EDTA per 1.0 mL of bead suspension) for 30 min with end-over-end rotation.
3. The EDTA solution was removed, and the beads were washed 3 \times with nano-pure water (800.0 μL of water per 1.0 mL of bead suspension).
4. Beads were treated with 10 mM aqueous metal-ion solution (800.0 μL of 100 mM FeCl_3 per 1.0 mL of bead suspension) for 30 min with end-over-end rotation.
5. After removing excess metal-ions, beads were washed 3 \times with water (800.0 μL of water per 1.0 mL of bead suspension), and resuspended in 1:1:1 acetonitrile/methanol/0.01% acetic acid for aliquotting into microcentrifuge tubes.

Phosphopeptide enrichment

A 50 μL aliquot of the 5% suspension metal-ion activated NTA was used per 100 μg of peptides.

Note: Tubes that are used to collect eluted phosphopeptides are pre-washed with 200 μL of acetonitrile for 20 min before elution.

6. Resuspend peptide sample in wash/resuspending buffer (80% acetonitrile, 0.1% TFA) – 100.0 μg in 200.0 μL of the buffer.
7. Wash beads with of 80% acetonitrile with 0.1% TFA (200 μL of 80% acetonitrile per 50.0 μL of beads). Discard wash buffer.
8. Add resuspended samples (100 μg peptides in 200 μL of 80% acetonitrile, 0.1% TFA) to the activated beads and incubate for 30 min with end-over-end rotation. *Collect and save supernatant for future reference if required.*

9. Wash beads for 1 min with 80% acetonitrile, 0.1% TFA (200 μ L of 80% acetonitrile per 50.0 μ L of beads), remove the supernatant.
10. Repeat wash step 3 \times more. Discard the liquid.
11. Elute phosphopeptides using appropriate amount of 1:1 acetonitrile/1:20 ammonia/water (50.0 μ L of elution buffer per every 50.0 μ L of beads/100.0 μ g of peptides).
12. Acidify final sample to pH 3.5-4.0 with 10% TFA, dry down (for 100.0 μ g of starting peptide sample) to a 5-10 μ L volume by vacuum centrifugation, and reconstitute to a final volume of 20-30 μ L with 0.1% TFA.

Modified protocol from the following reference:

Magnetic bead processor for rapid evaluation and optimization of parameters for phosphopeptide enrichment. Scott B. Ficarro, Guillaume Adelmant, Maria N. Tomar, Yi Zhang, Vincent J. Cheng, Jarrod A. Marto. **Analytical Chemistry**, 2009, *81* (11), 4566-4575.