STANDARD OPERATING PROCEDURE

Title: Peptide immunoaffinity enrichment by immobilized antibody on magnetic beads

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Purpose

The purpose of this document is to describe semi-automated enrichment of peptides using magnetic beads with immobilized anti-peptide antibodies.

Scope

This procedure may be used to enrich modified and nonmodified peptides out of digested complex biological matrix.

Responsibilities

It is the responsibility of person(s) performing this procedure to be familiar with laboratory safety procedures. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

Equipment

- KingFisher magnetic particle processor (Thermo Fisher, Waltham, MA).
- LabQuake tube rotator (Barnstead, ThermoFisher Scientific).
- Magnet (Thermo Fisher, A13346).
- 200 µL 96 well plates (ThermoFisher Scientific)
- PCR plates (BioRad, Hercules, CA)
- Square Matrix CapMats For 2 mL blocks (ThermoFisher Scientific)
- Chemically resistant sealing foil (BioExpress, Kaysville, UT)
- ThermalSeal® PCR Sealing Films (Genesee Scientific, San Diego, CA)

Materials

- GE Protein G Mag Sepharose, (GE Healthcare, 28-9513-79)

- phosphate buffered saline (PBS, ThermoFisher, #BP-399-20)
- CHAPS (ThermoFisher, #28300)
- Water (H2O), HPLC grade (Fisher, W5-1)
- Acetonitrile (ACN), HPLC grade (Fisher, A998-1)
- Acetic Acid (HOAc), (Sigma, 242853)
- Formic Acid (FA), (EDM, 11670-1)
- Citric Acid (Sigma C0706)

Solutions

- 1×PBS / 0.01% CHAPS in LCMS-grade water.
- 0.1×PBS / 0.01%CHAPS.
- Peptide elution solution: 3% v/v acetonitrile (ACN) / 5% v/v acetic acid (AcOH) / 50 mM citrate.
- Antibody stock solutions: Purified monoclonal antibody stocks from the vendor should be in 1×PBS / 0.1% sodium azide. Store at 4 °C (for long-term storage, use -20 °C or -80 °C).
- Antibody working master mix: Combine equivalent amounts of antibodies cross-linked onto magnetic beads, with a final concentration of 1 µg of each antibody per 50 µL. Store in 1×PBS / 0.03% CHAPS / 0.1% sodium azide at 4 °C.

Procedure

1. Resuspend the digested lysate in 200 µL of 1×PBS / 0.01% CHAPS.
2. Adjust the pH to between 7.5-8.0 with 1 M Tris, pH 8.0. Check pH by pipetting 1 µL onto a pH 5-10 test strip. Verify that pH is between 7.5-8.0 before continuing to addition of antibody-beads.
3. Transfer the resuspended digests to 200 µL 96 well plates (the plates actually hold up to 400 µL of liquid).
4. Add the Antibody working master mix to each sample well to deliver an equivalent of 1 µg of each antibody.
5. Cover the plate with a Square Matrix CapMat and seal it by pressure to ensure that no liquid can leak from any of the wells.
6. Incubate samples overnight at 4 °C with tumbling.
7. Remove samples from the tumbler and centrifuge at 800 × g for 30 seconds to remove liquid that may be on the mat surface. Then carefully remove the mat.
8. Wash the beads and elute the peptides.
    a. Wash the beads twice with 200 µL of 1×PBS / 0.01% CHAPS and once with 200 µL of 0.1×PBS / 0.01% CHAPS by mixing beads for 1.5 min in each wash solution and using a magnet to separate the beads from the supernatant in between washes.

   b. The peptides are eluted in PCR plates by mixing the beads for 5 min in 26 µL of Peptide Elution Solution (3% acetonitrile / 5% acetic acid / 50 mM citrate). Separate the beads from the eluate using a magnet.
9. Cover the plate containing the eluates with ThermalSeal® PCR Sealing Film and spin the plate at 800 × g for 30 seconds.
10. Place the elution plate on a plate magnet for 5 minutes.
11. Transfer and split the eluates to two clean PCR plates by carefully drawing up 2 × 12 µL of the supernatant using a multi-channel pipet, without touching the bottom or sides of the wells, and transferring into corresponding wells of two clean PCR plates. Cover one plate with chemically resistant sealing foil, spin down the plate briefly and store it at -80 °C.
12. If analyzing samples immediately, proceed to next step using the second plate. Otherwise, cover the plate with chemically resistant sealing foil, spin it down briefly, and store at -80 °C.

Referenced Documents

n/a