PROTOCOL

ELUTION FROM BEADS USING AMMONIUM HYDROXIDE

This protocol can be used to elute proteins off of beads prior to Mass Spectrometry Analysis. This is an alternative procedure to on-bead tryptic digestion.

Procedure:

1. Make fresh elution buffer:
   Elution buffer (final) – (5mL)
   9.5M NH_4OH, pH 11.0-12.0
   0.5mM EDTA
2. Wash beads 2X with lysis buffer (same lysis buffer that was used to lyse cells).
3. Wash beads 2-3X with the same lysis buffer but without detergents, which helps to remove incompatible detergents from sample prior to mass spectrometry analysis.
4. Elute proteins 2-3 times with three bead volumes of elution buffer @ 4ºC with end-over-end agitation for 15 minutes. It is recommended that the elution buffer is prepared fresh. Additionally, monitor the pH of the elution solution before use, pH should equal 11-12 for efficient protein elution. Alternatively, 2% formic acid (HCO_2H) can be used for elution however acid elution significantly increased the amount of antibody light and heavy chains in the eluate, and is not as volatile. Alternatively, peptides can be used for elution by competitive inhibition.
5. Combine Eluates and freeze on dry ice. Bring samples to SPARC facility for drying and endoproteinase digestion or continue to step 6.
6. Dry samples in speed-vacuum. Bring samples to SPARC facility for endoproteinase digestion or continue with in-solution digestion protocol.