PROTOCOL: Final Sample Resuspension (FSR)

Purpose

Prepare samples for mass spec analysis following the final desalt on the AssayMAP BRAVO. The addition of heavy synthetic peptides allow for QC during analysis, quantify phosopho-sites in endogenous samples, and compare phospho-mark abundance across sample sets.

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This protocol is to be followed the day samples are ready to be analyzed on the Q Exactive HF
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Preparation

1. Thaw Heavy Synthetic Peptide Mixture {FSR-M04} on ice
2. Remove dried, desalted, eluate from the freezer location. Bring to room temperature.

Materials

- Acetonitrile (ACN), EMD Millipore, Cat. No. AX0156-1 {FSR-M01}
- Formic Acid (FA), Sigma-Aldrich, Cat. No. S6302-50ML-F {FSR-M02}
- HPLC-grade water, JT Baker, Cat. No. 4218-03 {FSR-M03}
- Concentrated Heavy Synthetic Peptide Mixture {FSR-M04}
- Micronic Split-Septa TPE Push Caps, Micronic, Cat. No. 1775-3026 {FSR-M05}
- 1L Glass Graduated Cylinder {FSR-M06}
- 1L Glass Reagents Bottle {FSR-M07}
- 40 mL Amber Vial, Solid Screw Top, PTFE Liner {FSR-M08} [SUPLECO, 27182]
- 1.7 mL Microcentrifuge Tubes {FSR-M09} [Axygen, MCT-175-L-C]
- 0.5 mL Microcentrifuge Tubes {FSR-M10} [Axygen, MCT-060-L-C]

Assets

Reagent Mixes

ID	Name	Step	Composition	Stock Volume	Use
FSRMIX 01	3%ACN/5%FA	FSR	3% {FSR-M01} and 5% FA {FSR-M02} in {FSR-M03}	40 mL	Balance reagent in creation of heavy synthetic peptide mixture
FSR MIX02	1:50 Dilution of Concentrated Heavy Synthetic Phosphopeptide Mix {FSR-M04}	FSR	1:50 dilution of concentrated heavy phosphopeptides in 3%ACN/5%FA {FSRBUF01}	22uL at 50x concentration	Mixture of heavy synthetic peptides used as an internal standard in the analysis of samples

Preparation of Mixture Reagents:

FSRMIX01- 3% ACN/ 5% FA :

- ❏ Add 36.8 mL of HPLC-grade water {FSR-M03} to 40 mL amber vial with screw top{FSR-M10}
- ❏ Add 2 mL of formic acid {FSR-M02} to vial
- ❏ Add 1.2 mL of acetonitrile {FSR-M01} to vial
- ❏ Vortex to mix.

FSRMIX02- 1:50 Dilution of Heavy-tagged Phosphopeptide Standard Mix

- ❏ Add 1078uL of 3%ACN/5%FA {FSRMIX01} to 22uL of Concentrated Heavy-tagged Phosphopeptide Standard Mix {FSR-M04}.

Procedure

1. Deliver 10uL of FSRMIX02 to each sample.
2. Cover the individual sample vials with split-septa TPE push caps {FSR-M05}.
3. Spin down the plate.
4. Keep plate at 4℃ until it is ready to be placed on the the deck of the HPLC system.