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Proteomic Sample Preparation

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Shiyi Wang[1]

[1]Duke University

Shiyi Wang
Duke University

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We use this protocol and it's working

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Abstract

Proteomic Sample Preparation

1 **Sample Storage and Preparation** - Received 12 samples (3 of each WT CYT, GS CYT, WT EZR, and GS EZR) and kept at -80°C until processing. - Spike samples with undigested bovine casein at a total of either 1 or 2 pmol as an internal quality control standard.

2 **Reduction and Alkylation** - Reduce samples for 15 minutes at 80°C. - Alkylate with 20 mM iodoacetamide for 30 minutes at room temperature.

3 **Protein Trapping** - Supplement samples with a final concentration of 1.2% phosphoric acid and 636 µL of S-Trap (Protifi) binding buffer (90% MeOH/100 mM TEAB). - Trap proteins on the S-Trap micro cartridge.

4 **Digestion** - Digest trapped proteins using 20 ng/µL sequencing grade trypsin (Promega) for 1 hour at 47°C.

5 **Elution** - Elute digested proteins using the following solutions in sequence: - 50 mM TEAB - 0.2% FA - 50% ACN/0.2% FA

6 **Lyophilization** - Lyophilize all samples to dryness.

7 **Resuspension** - Resuspend samples in 12 µL of 1% TFA/2% acetonitrile with 12.5 fmol/µL of yeast ADH.

8 **Study Pool QC (SPQC) Creation** - Create a study pool QC (SPQC) by combining equal volumes of each sample.