PROTOCOL: GCP Protein Desalt (OASIS)

Purpose

To remove salts from the samples before trypsin digest.

Preparation

1. Prepare reagents as needed

Materials

- HPLC-grade water {OASIS-M01} [JT Baker, 4218-03]
- Acetonitrile (ACN) {OASIS-M02} [EMD Millipore, AX0156-1]
- Trifluoroacetic Acid (TFA) {OASIS-M03} [Sigma-Aldrich, T6508-25ML]
- Oasis HLB 5mg Plate {OASIS-M04} [Waters, 186000309]
- 500uL V-bottom plate {OASIS-M05} [VWR, 89005-016]
- Axygen -80°C Rated Foil Seal {OASIS-M06} [Axygen, PCR-AS-200]
- Breath-EASIER seal {OASIS-M07} [BERM-2000]

Assets

- 96-well Positive Pressure Desalting Platform {OASIS-A01}
- 12-channel Multichannel pipette {OASIS-A02}
- Desalting flow through waste plate {OASIS-A04}
- Thermo Scientific Savant SC210A Concentrator {OASIS-A05}

Reagent Mixes

ID	Name	Step	Composition	Volume/Well	Use
MIX01	20% ACN/0.1% TFA	**OASIS**	20% acetonitrile {OASIS-M02}/0.1 % TFA { OASIS-M03} in HPLC-grade water {OASIS-M01}	200uL	Used to equilibrate the Oasis cartridge plate, and wash samples once they have been loaded onto the plate
MIX02	60% ACN/0.1% TFA	**OASIS**	60% acetonitrile {OASIS-M02}/0.1 % TFA { OASIS-M03} in HPLC-grade water {OASIS-M01}	100uL	Used to elute desalted samples from the Oasis plate

Mix Preps and Mini-worksheets:

MIX01 – 20% ACN/0.1% TFA

1. Measure 800mL of HPLC-grade water {OASIS-M01} in a graduated cylinder and add to a 1L bottle. ❑
2. Measure 200mL of acetonitrile {OASIS-M02} in a graduated cylinder and add to the bottle. ❑
3. Pipette 1mL Trifluoroacetic acid {OASIS-M03} into the bottle. ❑

MIX02 – 60% ACN/0.1% TFA

1. Measure 400mL of HPLC-grade water {OASIS-M01} in a graduated cylinder and add to a 1L bottle. ❑
2. Measure 600mL of acetonitrile {OASIS-M02} in a graduated cylinder and add to the bottle. ❑
3. Pipette 1mL Trifluoroacetic acid {OASIS-M03} into the bottle. ❑

Procedure

1. Place the Oasis HLB plate {OASIS-M04} on top of flow-through plate {OASIS-A04}.
2. Activate wells of OASIS plate with 200 uL of 100% acetonitrile {OASIS-M02}. Let this step flow through via gravity alone.
3. Equilibrate the wells of the Oasis plate with 200 uL of 20% ACN/0.1% TFA {MIX01}.
4. Repeat step 4.

NB: At this point check volume in waste plate. Discard waste in liquids waste bucket on the bench.

5. Load sample onto the Oasis plate (375uL/sample). With the multichannel pipette {Oasis-A02} mix samples 4 times, and on the 5th time draw up sample and load onto the Oasis plate. Draw up remaining volume and dispense into Oasis plate.
6. Wash Oasis wells with 200 uL of 20% ACN/0.1% TFA {MIX01}.
7. Repeat step 7.
8. Remove waste plate from the Oasis desalting assembly and attach the elution plate {OASIS-M05}. Elution plate should be labeled with name, date, and study.
9. Elute samples with 100 uL of 60% ACN/0.1% TFA {MIX02}. Let this step flow through via gravity, with as minimal assistance from the Positive Pressure Desalting Platform {OASIS-A01} as possible. When the wells look dry, slowly dial up the pressure applied by the desalting platform {OASIS-A01}, until the rest of the volume passes into the elution plate.
10. Remove the Oasis plate from the sample elution plate, seal the elution plate with a foil seal {OASIS-M06}, and freeze at -80℃.
11. Create a balance plate of 60% ACN/ 0.1%TFA {MIX02} in the same plate type as elution plate. Seal and freeze at -80℃ as well.
12. Once plates are frozen, remove foil seal and replace with Breath-EASIER seal {OASIS-M07}.
13. Once both sample and balance plate are frozen, speedvac {OASIS-A05} to dryness.