

PROTOCOL: Post Desalt Sample Resuspension (RES)

Purpose

Resuspend dried peptides in 80% acetonitrile/0.1% Trifluoroacetic acid and spike in medium tagged phosphopeptide standards to track IMAC enrichment efficiency.

NB: Samples should be resuspended while the **TIP** protocol is running.

NB: Keep samples on ice until transferring to **IMAC** protocol.

Preparation

1. Prepare RESMIX01 (50% ACN/0.1% TFA)
2. Prepare RESMIX02 (100% ACN/0.1% TFA)
3. Prepare RESMIX03 (80% ACN/0.1% TFA)
4. Prepare RESMIX04 (Phosphopeptide Standards)

Materials

- HPLC-grade water, JT Baker, Cat. No. 4218-03 {RES-M01}
- Acetonitrile, EMD Millipore, Cat. No. AX0156-1 {RES-M02}
- Trifluoroacetic acid (TFA), Sigma-Aldrich, T6508-25ML {RES-M03}
- Concentrated Medium-tagged Phosphopeptide Standard Mix {RES-M04}
- 96-Well Round Bottom Microplate, Greiner Bio-One, Cat. No. 650101 {RES-M05} [650101]

Assets

- Sonicator {RES-A01}

Reagent Mixes

ID	Name	Step	Composition	Volume/Well	Use
RESMIX01	50% ACN/0.1% TFA	RES	50% acetonitrile {RES-M02}/0.1% TFA {RES-M03} in HPLC-grade water {RES-M01}	100uL	Resuspend dried sample peptides.
RESMIX02	100% ACN/0.1% TFA	RES	100% acetonitrile {RES-M02}/0.1% TFA {RES-M03} in HPLC-grade water {RES-M01}	140uL	Adjust acetonitrile concentration of resuspended peptide solutions to 80%.

RESMIX03	80% ACN/0.1% TFA	RES	80% acetonitrile {RES-M02}/0.1% TFA {RES-M03} in HPLC-grade water {RES-M01}	N/A	Create 1:50 dilution of medium phosphopeptides.
RESMIX04	1:50 Dilution of Concentrated Medium-tagged Phosphopeptide Standard Mix {RES-M05}	RES	1:50 dilution of concentrated medium phosphopeptides in 80% ACN/0.1% TFA {RESMIX03}	10uL	Track enrichment efficiency of IMAC cartridges.

Mix Preps and Mini-worksheets:

RESMIX01 – 50% ACN/0.1% TFA

1. Measure 500mL of HPLC-grade water {RES-M01} in a graduated cylinder and add to a 1L bottle.
2. Measure 500mL of acetonitrile {RES-M02} in a graduated cylinder and add to the bottle.
3. Pipette 1mL Trifluoroacetic acid {RES-M03} into the bottle.

RESMIX02 – 100% ACN/0.1% TFA

1. Measure 999mL of acetonitrile {RES-M02} in a graduated cylinder and add to a 1L bottle.
2. Pipette 1mL Trifluoroacetic acid {RES-M03} into the bottle.

RESMIX03 – 80% ACN/0.1% TFA

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

RESMIX04 – 1:50 Dilution of Medium-tagged Phosphopeptide Standard Mix

1. Add 1078uL of 80% ACN/0.1% TFA {RESMIX03} to 22uL of Concentrated Medium-tagged Phosphopeptide Standard Mix {RES-M04}.

Procedure

1. Add 100uL of 50% ACN/0.1% TFA to each dried peptide sample.
 - 1.1. Cover plate with foil, sonicate for 10 minutes, vortex thoroughly, and spin down.
2. Add 140uL of 100% ACN/0.1% TFA to each sample.
 - 2.1. Cover with foil, sonicate for 10 minutes, vortex thoroughly, and spin down.

- 2.2. Samples will now be in 80% ACN/0.1% TFA.
3. Use a multichannel pipette to transfer each sample to a 96-well round bottom microplate {RES-M05}.
 - 3.1. The maximum volume this plate can hold is 250uL per well; samples should be 240uL each.
4. Add 10uL of diluted medium-tagged phosphopeptide standards in 80% ACN/0.1% TFA {RESMIX04} to each sample using a manual pipette.
 - 4.1. Spin plate to mix phosphopeptides thoroughly.

NB: Use caution while handling the plate. Do not seal the plate with foil.

5. Place plate at 4°C until ready to continue to **IMAC** enrichment.