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Protein Aggregation Capture

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

PAC Adapted from Batth et al., 2019

Protocol materials

Tris **P212121** Step 10

TFA **Thermo Scientific** In 2 steps

MagReSyn Hydroxyl Beads **Resyn Biosciences** Step 1

Acetonitrile Step 5

70% ACN **Thermo Scientific** In 2 steps

100% ACN **Thermo Scientific** Step 8

70% Ethanol Step 9

Trypsin **P212121** **Catalog #**RP-T70010 Step 11

Equilibration

1 Add 5 µL of MagReSyn Hydroxyl Beads **Resyn Biosciences** to 1.5mL LoBind Eppendorf tube

1.1 Place tube on magnet and allow Microparticles to clear 00:00:10 10s

1.2 Remove storage solution and discard

2 Equilibrate Microparticles by adding 100 µL 70% ACN **Thermo Scientific** and mix by gentle agitation

2.1 Place tube on magnet and allow Microparticles to clear 00:00:10 10s

2.2 Remove supernatant and discard

3 Add 100 µL 70% ACN **Thermo Scientific** and mix by gentle agitation

3.1 Place tube on magnet and allow Microparticles to clear 00:00:10 10s

3.2 Remove supernatant and discard

4 Add 2 µL sample in triplicate

5 Add Acetonitrile **Contributed by users** to a concentration of 70% and mix once by pipette to create a uniform suspension

6 Allow to rest 00:10:00 — 10m

Wash - DO NOT REMOVE FROM MAGNET — 10s

7 Place tube on magnet and allow Microparticles to clear 00:00:10 — 10s

7.1 Remove supernatant and discard

8 Add 1 mL 100% ACN **Thermo Scientific** and incubate for 00:00:10 — 10s

8.1 Remove supernatant

9 Add 1 mL 70% Ethanol **Contributed by users** and incubate for 00:00:10 — 10s

9.1 Remove supernatant

Digestion — 10s

10 Remove tube from magnet and add pH 8.5 Tris **P212121** and mix throughly

11 Add 5 µg Trypsin **P212121 Catalog #**RP-T70010 and incubate for 04:00:00 — 4h

12 Quench to a final concentration [M] 1 % volume TFA **Thermo Scientific**

13 Mix and place samples on magnet for 00:01:00 and transfer supernatant to tubes — 1m

14 Wash beads with 50-100 µL [M] 1 % volume TFA **Thermo Scientific** for 00:02:00 with continous mixing — 2m

14.1 Remove supernatant and pool with eluate from Step 13

15 Centrifuge tubes 20000 x g, 00:10:00 10m

16 Transfer supernatant to new tube and freeze down at -80 °C