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Proteomic Analysis of Human Ovarian Cortex and Medulla Secretome Using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Acquisition by Data-Independent Acquisition (DIA) on an Orbitrap Eclipse Tribrid Mass Spectrometer

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Metabolomics Protocols ... Cellular Senescence Net...

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We use this protocol and it's working

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Abstract

Ovaries from human donors were cut into 3-5 mm sections. These sections were further processed into 500 µm slices containing cortex and medulla. The slices were then processed into pieces (1 mm x 1 mm x 500 µm), and cortex and medulla pieces were cultured separately as explants in static cultures.
Explants were cultured and treated with either DMSO vehicle control or 0.1 µg/mL doxorubicin to induce senescence. After 10 days, cortex and medulla explants were thoroughly washed with serum-free basal media and transferred to a clean plate with pre-equilibrated serum-free basal media and inserts. The conditioned media were collected after 24 hours for secretome proteomics profiling.

The concentrated conditioned media was subjected to tryptic digestion using S-trap Spin columns. The reconstituted peptide elution was desalted with C18 hydrophilic-lipophilic balance (HLB) cartridges. The final reconstituted peptides were diluted with 2% ACN and 0.1% FA. Proteolytic peptide measurement was completed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) acquisition by Data-Independent Acquisition (DIA) on an Orbitrap Eclipse Tribrid mass spectrometer for peptide/protein identification and quantification.

Troubleshooting

Conditioned Media Concentration with Amicon Ultra Centrifugal Filters

1 dx.doi.org/10.17504/protocols.io.e6nvw1px7lmk/v1

Protein Digestion with S-trap Spin Columns using Conditioned Concentrated Media

2 dx.doi.org/10.17504/protocols.io.x54v928eml3e/v1

Proteolytic Peptide Desalting with C18 HLB Cartridges

3 dx.doi.org/10.17504/protocols.io.eq2lywdzpvx9/v1

LC-MS/MS Acquisition by DIA on an Orbitrap Eclipse Tribrid Mass Spectrometer

4 dx.doi.org/10.17504/protocols.io.36wgq3m15lk5/v1

DIA Data Processing using Spectronaut/directDIA (Biognosys): Secretome Analysis

5 dx.doi.org/10.17504/protocols.io.q26g71rz3gwz/v1