Proteomics Analysis

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Abstract

This protocol provides an efficient and standardized way to prepare samples ready for mass spec analysis.

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Before start

Prepare solutions as described in the steps.

Protocol

Sample preparation

Step 1.

Dilute the amount of protein needed, taking into account complexity, into 25mM AmBic. Small Eppendorf tube preferred.

Detergent treatment

Step 2.

Add 10µL of 1% (w/v) RapiGest (0.05% (w/v) final).

AMOUNT

10 µl Additional info: 1% (w/v) RapiGest

Detergent treatment

Step 3.

Heat at 80°C, 10minutes, vortex briefly at 5min.

TEMPERATURE

80 °C Additional info: Heat

Detergent treatment

Step 4.

Spin quickly to return liquid to the bottom of the tube.

Reduction

Step 5.

Add 10 µL of a 9.2mg/mL solution of DTT (3 mM final). Vortex mix.

AMOUNT

10 µl Additional info: 9.2mg/mL solution of DTT

Reduction

Step 6.

Incubate for 60°C, 10minutes.

TEMPERATURE

60 °C Additional info: Incubation

Reduction

Step 7.

Cool to RT and quickly spin to return liquid to the bottom of the tube.

Alkylation

Step 8.

Add 10 µL of a 33mg/mL solution of iodoacetamide (9 mM final). Vortex.

AMOUNT

10 µl Additional info: 33mg/mL solution of iodoacetamide

Alkylation

Step 9.

Incubate at RT, **IN THE DARK** for 30min.

Digestion

Step 10.

Add trypsin to 50:1 protein:trypsin ratio. Incubate 12-16h (overnight) at 37°C.

TEMPERATURE

37 °C Additional info: Incubation

Mass spec analysis

Step 11.

Analyze peptide mixtures on either nanoACQUITY-nLC system (Waters MS technologies) or Ultimate 3000 nano system (Thermo Fisher Scientific) followed by an LTQ-Orbitrap Velos (ThermoFisher Scientific) mass spectrometer or a Q-Exactive mass spectrometer (Thermo Fisher Scientific).