

iTRAQ Procedure



Note: * In case of membrane protein analysis, chymotrypsin is also applied in the second day of digestion.

** Isopropanol is used instead of ethanol when iTRAQ 8-plex is performed.

Cells extraction and preparation for iTRAQ analysis

Cells

- Wash twice with cold water

Extract

- Buffer:* 43 mM NaCl, 81 mM MgSO₄, 27 mM KCl
- Ultrasonicate at duty cycle 70% for 1min sec then leave on ice for 1 min. Repeat 3-4 times.

Low speed centrifuge

- 3,000g for 5 min
- Discard unbroken cells and debris

Ultra speed centrifuge

- 100,000g for 90 min
- Collect pellet as a membrane-enriched fraction

Delipidate membrane

- Cloroform/methanol are used to remove lipid

Resuspend in iTRAQ buffer

- 20 μ L of TEAB pH8.5 and 1 μ L of 2%SDS

iTRAQ analysis

- Refer to iTRAQ protocol

SCX

- Strong cation exchange is used
- To clean and fractionate samples
- Buffer A: 10 mM KHPO₄ + 25% ACN
- Buffer B: 10 mM KHPO₄ + 25% CAN + 0.5 M KCl

Mass spectrometry

- NanoLC-ESI-qQ-TOF
- ESI-ionTrap-CID/ETD-MS

Analyse data based on Protein Pilot or Phenyx

- Note: for soluble fraction, buffer TEAB is used and delipidate step is omitted

Results