

iTRAQ Labeling Procedure

05/08/07

Reagents

All Reagents part of iTRAQ kit from Applied Biosystems Cat # 4352135, except Trypsin

Procedure

NOTE: 1 vial of iTRAQ reagent labels up to 100 μ g of protein

Add 20 μ L of Dissolution Buffer to each dried protein sample

Add 1 μ L of Denaturant to sample

Vortex/Spin sample

Add 2 μ L of Reducing Reagent

Vortex/Spin sample

Incubate at 60°C for 1 hour

Spin sample briefly

Add 1 μ L of Cysteine Blocking Reagent to sample

Vortex/Spin sample

Incubate at room temperature for 10 min

Add 25 μ L HPLC Grade H₂O to a vial of Trypsin (will need more than 1 vial for 3+ samples)

Add 10 μ L of Trypsin solution to each sample

Incubate at 37°C for 12 -16 hours

Spin sample briefly

Bring 1 vial of iTRAQ reagent to room temperature for every sample (different MW vial for each sample!)

Add 70 μ L of EtOH to each iTRAQ reagent vial

Add the iTRAQ reagent solution to each corresponding vial.
Vortex/Spin sample

Incubate at room temperature for 1 hour

Combine individual labeling reactions into a single vial

Dry down in Speed Vac in preparation for MCX cleanup