

# IC3/IC5-Labeling of Proteins Extracted from Cells

Cell Pellet (W mg)

← (W x 4)  $\mu$ l of Protein Extraction and IC-Labeling Buffer

Urea	0.48 g
10%(w/v) SDS	0.02 ml
20%(v/v) Triton X-100	0.10 ml
200 mM HEPES, pH 8.0	0.05 ml
Mill-Q water up to total	1.00 ml

↓ Ultra-sonicate

↓ Centrifuge at 15,000 x g for 15 min at 20°C

Supernatant (Protein sample solution)



(An aliquot containing 0.2 mg of protein)



← 10 volumes of acetone

↓ Vortex and chilled in a freezer at -20°C



↓ Centrifuge at 15,000 x g for 15 min at 4°C

Precipitate



← 20  $\mu$ l of Protein Extraction and IC-Labeling Buffer  
Resolubilization



← 2  $\mu$ l of 0.4 mM IC3/IC5-OSu  
Incubate at R.T. for 15 min in the dark



← 2  $\mu$ l of 10 mM ethanolamine  
Incubate at R.T. for 15 min in the dark



Combine IC3-labeled and IC5-labeled Samples



← 10 volumes of acetone  
Vortex and chilled in a freezer at -20°C



↓ Centrifuge at 15,000 x g for 15 min at 4°C

Precipitate



← 20  $\mu$ l of 2-DE Sample Buffer  
Dissolve



2-D Electrophoresis