## IC3/IC5-Labeling of Proteins Extracted from Cells

Cell Pellet (W mg) (W x 4) µl of Protein Extraction and IC-Labeling Buffer Urea 0.48 g10%(w/v) SDS 0.02 ml 20%(v/v) Triton X-100 0.10 ml 200 mM HEPES, pH 8.0 0.05 ml Ultra-sonicate Mill-Q water up to total 1.00 ml 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 cold acetone at -20°C Vortex and chilled in a freezer at -20°C for more than 30 min Centrifuge at 15,000 x g for 15 min at 4°C Precipitate 20 µl of Protein Extraction and IC-Labeling Buffer Resolubilization 2 µl of 0.4 mM IC3/IC5-OSu Incubate at R.T. for 15 min in the dark 2 µl of 10 mM ethanolamine-HCl,pH 8.0 Incubate at R.T. for 15 min in the dark Combine IC3-labrled and IC5-labeled Samples 10 volumes of cold acetone at -20°C Vortex and chilled in a freezer at -20°C for more than 30 min Centrifuge at 15,000 x g for 15 min at 4°C Precipitate 20 µl of 2-DE Sample Buffer Dissolve 2-D Electrophoresis

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