

Quantitative Protein Expression Technologies

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Introduction

Invitrogen offers a complementary suite of technologies that allow powerful differential protein expression analysis using mass spectrometry. Each technology is uniquely designed to address specific experimental needs such as sample source (tissue or cell culture), temporal analyses, reproducibility of sample preparation, multiplexing for sample analysis such as sample nativity for sub-cellular fractionation, experimental replicates, or a streamlined workflow.

Based on your experimental design, choose from the following quantitative protein expression technologies:

- iTRAQ™ Reagent Technology
- SILAC Metabolic Labeling Technology
- 1-D PAGE Cleavable ICAT® Reagent Technology

An overview of each protein tagging technology is described below and an experimental workflow for each technology is described on the following pages. For more details, refer to the manual supplied with each kit or contact Technical Service.

iTRAQ™ Reagent Technology

The iTRAQ™ Reagent Technology is an amine-specific peptide-based labeling technology for simultaneous identification and quantitation of biological samples. The multiplexing capability of the four isobaric iTRAQ™ reagents allows rapid, systematic and effective comparison of up to four independent samples using the iTRAQ™ Reagent 114, iTRAQ™ Reagent 115, iTRAQ™ Reagent 116, and iTRAQ™ Reagent 117. The iTRAQ™ Reagents are non-polymeric, isobaric (same mass) tagging reagents consisting of a reporter group, a balance group, and a peptide reactive group which covalently links the reagent with each lysine side chain and N-terminal group of a peptide. The iTRAQ™ Reagents when combined with Applied Biosystem's mass spectrometry instrumentation and application specific software, provides an integrated solution for biomarker quantitation and identification.

SILAC Metabolic Labeling Technology

The SILAC (Stable Isotope Labeling by Amino Acids in Cell Culture) Metabolic Labeling Technology is a powerful tool to identify and quantitate complex protein samples in mammalian cells. The SILAC Technology utilizes stable isotopic labeled amino acids in cell culture which when combined with global, differential mass spectrometric (MS) analysis provides a tool for quantitative analysis of post-translational modifications, low abundance proteins, phosphoproteins, and membrane proteins.

The SILAC Technology is ideal for quantitative analysis of differential protein expression in the presence of a stimulus or in response to stress, performing proteomic profiling in normal and diseased cells, or identifying inducible protein complex components.

Cleavable ICAT® Reagent Technology

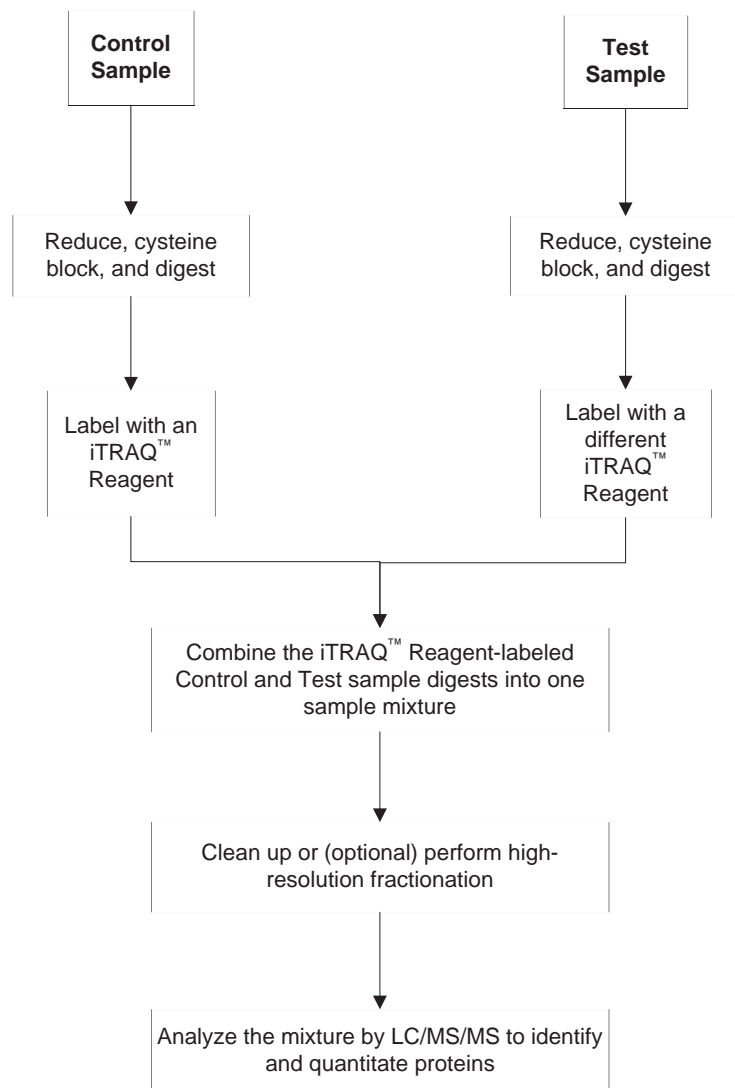
The 1-D PAGE (one-dimension polyacrylamide gel electrophoresis) Cleavable ICAT® Reagent Technology is a cysteine-specific protein-based labeling technique that combines one-dimension SDS-PAGE, isotope-coded affinity tag chemistry, and cleavable-linker technology to facilitate the isolation, identification and quantitation of differentially expressed proteins. Analyzing a Control Sample, (for example, a normal cell state) and a Test Sample (for example, a diseased cell state) produces ratios of ICAT® Reagent-labeled peptides that allow you to determine protein expression levels.

The Cleavable ICAT® Reagents combined with 1-D PAGE are ideal for investigating a protein or protein class with a known molecular weight range or for fractionating a complex protein sample prior to enzymatic digestion.

iTRAQ™ Reagent Technology

To perform the iTRAQ™ Reagent protocol, you will denature, reduce, and label each sample in a single tube. Then combine all iTRAQ™ Reagent-labeled samples into one sample mixture and analyze samples by LC/MS/MS for protein identification and quantitation.

The flowchart below summarizes the iTRAQ™ Reagent protocol for a duplex-type experiment. Using the iTRAQ™ Reagent protocol, you can prepare and analyze up to 4 samples in a single experiment. A list of User Supplied Materials is also included below. For a detailed protocol, refer to the manual supplied with the kit.



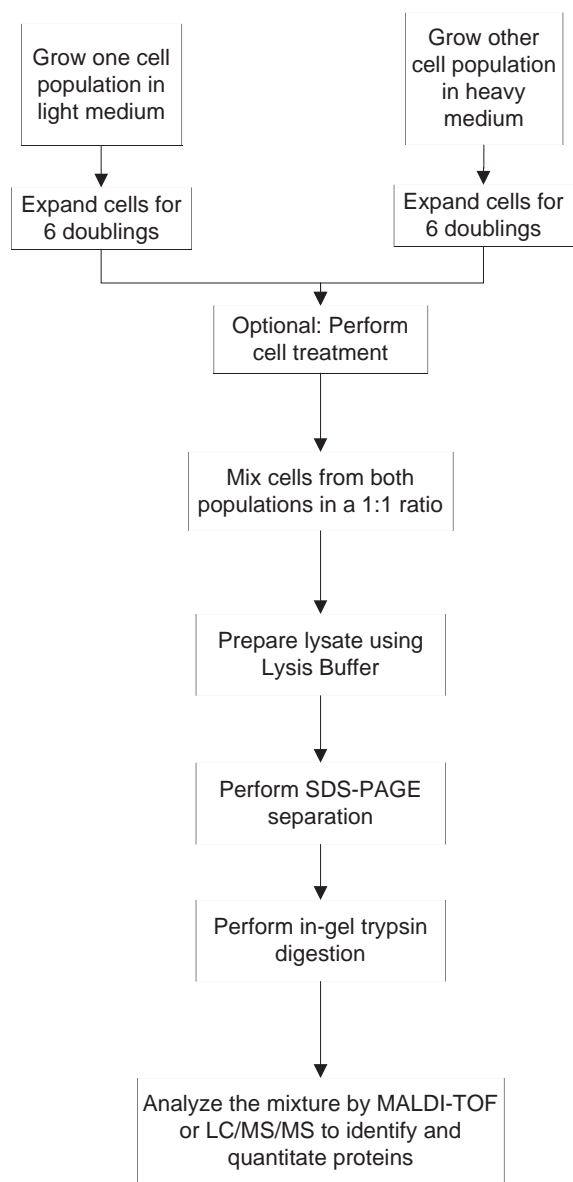
User Supplied Materials

- Control Protein Sample (normal cell state sample)
- Test Protein Sample (diseased cell state sample; up to 3 samples)
- Trypsin with CaCl₂ (cat. no. MS10015)
- Ultrapure water (minimum 18.2 MOhms water, conductivity maximum 0.05 µS/0.05 Mho)
- *Optional:* High-resolution cation exchange column (see manual for details)
- Fraction collection tubes and rack
 - 0.5-2.0 ml screw or snap-cap tubes
 - 1.5 ml and 4 ml tubes for cation exchange chromatography
- pH paper, (pH 2.5-4.5)
- Heating block, 60°C
- Incubator, 37°C
- Vortex
- Centrifuge
- Capillary reverse phase HPLC system
- Mass spectrometer with analysis software such as Applied Biosystems/MDS SCIEX QTRAP® System with ProQUANT Software (see manual for details)
- Disposable gloves, a 2.5 ml syringe (2-inch, blunt needle, 22 gauge), and pipettors with tips for 1 µl to 1 ml

SILAC Metabolic Labeling Technology

The SILAC Metabolic Labeling protocol involves metabolically labeling one cell population using non-radioactive isotopic labeled essential amino acid (heavy amino acid) and labeling the second cell population with normal essential amino acid (light amino acid) during cell culture. Then harvest cells from each cell population and mix cells from each cell population using a 1:1 ratio based on cell number. Lyse the cells, perform SDS-PAGE, and in-gel trypsin digestion. Analyse the tryptic peptides by MALDI-TOF MS or LC/MS/MS for protein identification and quantitation.

The flowchart below summarizes the SILAC Metabolic Labeling protocol. A list of User Supplied Materials is also included below. For a detailed protocol and application specific protocols (phosphoprotein or membrane protein analysis), refer to the manual supplied with the kit.



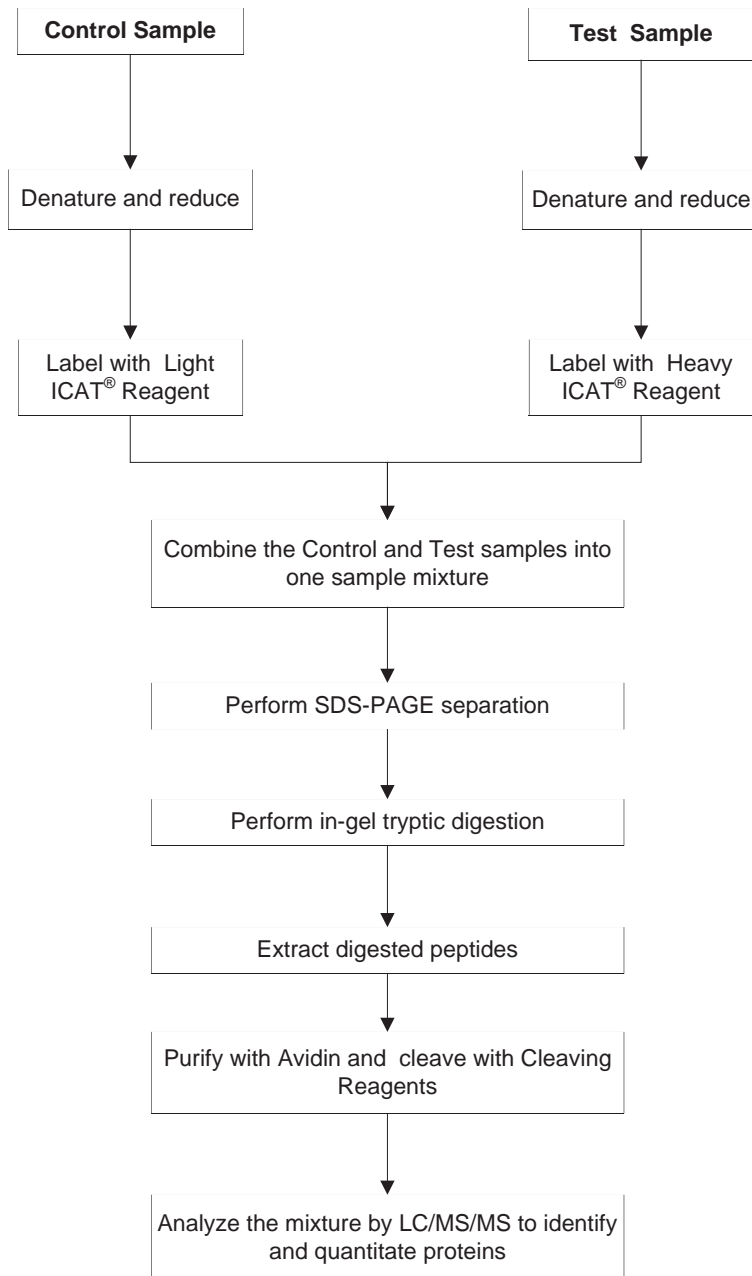
User Supplied Materials

- Mammalian cell line of choice
- Antibiotics (penicillin, streptomycin; cat. no. 15070-063)
- *Optional:* appropriate growth factors needed for your cells
- Appropriate tissue culture dishes and flasks
- 37°C incubator with a humidified atmosphere of 8% CO₂
- Reagents to determine viable and total cell counts (cat. no. 15250-061)
- 0.45 µm filtration unit to filter sterilize the medium
- *Optional:* reagents for cell treatment
- Trypsin with CaCl₂ (cat. no. MS10015)
- 25 mM ammonium bicarbonate, pH 8.0 for trypsin digestion
- 100% acetonitrile
- Ultrapure water (minimum 18.2 MOhms water, conductivity maximum 0.05 µS/0.05 Mho)
- NuPAGE® Novex Bis-Tris Mini Gels
- NuPAGE® LDS Sample Buffer (cat. no. NP0007), NuPAGE® MOPS/MES SDS Running Buffer (cat. no. NP0001/NP0002), and NuPAGE® Sample Reducing Agent (cat. no. NP0004)
- XCell SureLock™ Mini-Cell for gel electrophoresis (cat. no. EI0001)
- Protein stains (SimplyBlue™ SafeStain; cat. no. LC6060 or SilverQuest™ Silver Staining Kit; cat. no. LC6070)
- Clean single-edged razor blade or scalpel to excise gel bands
- Heating block, 70°C
- Vortex
- Centrifuge
- Centrifugal vacuum concentrator
- Mass spectrometer (for example, AB/MDS Sciex Family of MALDI-TOF/TOF™ Analyzers with the GPS Explorer™ 3.x software)
- Disposable gloves and pipettors with tips for 1 µl to 1 ml and gel loading tips

Cleavable ICAT® Reagent Technology

To perform the 1-D PAGE Cleavable ICAT® Reagent protocol, you will denature, reduce, and label the control and test samples. Then combine the control and test samples into one mixture, perform SDS-PAGE and in-gel trypsin digest followed by affinity purification of the ICAT® labeled peptides and cleavage of the biotin tag. The resulting samples are analyzed by LC/MS/MS for protein identification and quantitation.

The flowchart below summarizes the 1-D PAGE Cleavable ICAT® Reagent protocol. A list of User Supplied Materials is also included below. For a detailed protocol, refer to the manual supplied with the kit.



User Supplied Materials

- Control Protein Sample (normal cell state sample)
- Test Protein Sample (diseased cell state sample; up to 3 samples)
- Tris-Glycine SDS Sample Buffer (cat. no. LC2676), Tris-Glycine SDS Running Buffer (cat. no. LC2675), and NuPAGE® Sample Reducing Agent (cat. no. NP0004)
- XCell SureLock™ Mini-Cell for gel electrophoresis (cat. no. EI0001)
- Ultrapure water (minimum 18.2 MOhms water, conductivity maximum 0.05 µS/0.05 Mho)
- SimplyBlue™ SafeStain (cat. no LC6060)
- Gel Dehydration Solution (100% acetonitrile)
- Gel Washing Buffer (50% acetonitrile, in 0.1 M ammonium bicarbonate, pH 8.0)
- Extraction Solvent (50% acetonitrile containing 0.1% TFA)
- 0.1 M ammonium bicarbonate, pH 8.0 to reconstitute trypsin
- Fraction collection tubes and rack
- 2 ml screw cap and 1.5 ml eppendorf tubes
- pH paper, (pH 6-8)
- Clean single-edged razor blade or scalpel to excise gel bands
- Clean container for gel destaining
- Sonic water bath to extract peptides from the gel
- Heating block, 60°C and 100°C
- Incubator, 37°C
- Vortex
- Platform rocker for gel destaining
- Centrifuge
- Centrifugal vacuum concentrator
- Mass spectrometer with ICAT® software (see manual for details)
- Capillary reverse phase HPLC system
- Disposable gloves, a 2.5 ml syringe (2-inch, blunt needle, 22 gauge), and pipettors with tips for 1 µl to 1 ml and gel loading tips