Multiplexed Relative and Absolute Protein Quantitation Chemistry Quick Reference Card

This Quick Reference Card provides abbreviated procedures you can refer to when you use the iTRAQ® Reagents – 8plex One Assay or Multi-Plex Kits. For general chemical safety information, background information, a list of components required to perform the protocol, and detailed procedures, refer to the *iTRAQ® Reagents – 8plex Protocol*.

Refer to the *xTRAQ Family of Amine -Modifying Labeling Reagents for Multiplexed Relative and Absolute Quantification: Chemistry Reference Guide* for supplementary information on iTRAQ Reagent chemistry.

For the portable document format (PDF) versions of the protocol (PN 4375249), chemistry reference guide (PN 4351918), and this quick reference card (PN 4383502), go to http://www.sciex.com.

Safety

For safety and biohazard guidelines, refer to the "Safety" section in the *iTRAQ® Reagents – 8plex Protocol*. For all chemicals in bold red type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Preparing Sample

Make sure your samples are dry or concentrated and are free of thiols, high-detergent or denaturant concentrations, active proteases, or primary amines (other than the analyte of interest). Primary amines can react with iTRAQ Reagents – 8plex, competing with peptide derivitization. If necessary, clean up the samples using acetone precipitation. See the *iTRAQ® Reagents – 8plex Protocol,* Chapter 2, "Before You Begin", for details on potentially interfering substances and sample clean up.

Testing the Protocol

To verify that your sample preparation protocol does not interfere with the iTRAQ Reagents – 8plex labeling, it is recommended that you run a control sample through the entire protocol before you run an actual experiment.

If you lack enough control sample to test the protocol, prepare a sample using your sample conditions and the 6-Protein Mix in the One Assay kit. Label the 6-Protein Mix sample with one of the iTRAQ Reagents – 8plex. For more information about using the 6-Protein Mix and guidelines for modifying the protocol, see the *iTRAQ® Reagents – 8plex Protocol,* and the *xTRAQ Family of Amine -Modifying Labeling Reagents for Multiplexed Relative and Absolute Quantification: Chemistry Reference Guide.*

Running the Protocol

Follow the procedures shown on page 2. Modify the procedures if, when testing the protocol, you determine that alternative steps are required for your sample.

IMPORTANT! For optimal efficiency during labeling, the pH of the mixture must be greater than 7.5. If the pH is less than 7.5, labeling efficiency can be significantly reduced. Be sure to check and adjust pH as directed in section C, step 3c.

Preparing the iTRAQ® Reagents-Labeled Sample Mixture for LC/MS/MS Analysis

Some substances (such as SDS, buffer salts, and high concentrations of organic solvents) in an iTRAQ Reagents – 8plex-labeled sample mixture may interfere with LC/MS/MS analysis. Before LC/MS/MS analysis, clean up the sample mixture using cation-exchange chromatography (simple mixtures) or high-resolution cation-exchange chromatography (complex mixtures). See the *xTRAQ Family of Amine -Modifying Labeling Reagents for Multiplexed Relative and Absolute Quantification: Chemistry Reference Guide.*

Analyzing the iTRAQ® Reagents – 8plex-Labeled Sample Mixture

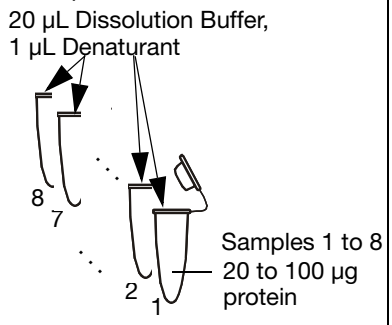
For information on electrospray and MALDI LC/MS/MS analysis using an AB SCIEX 5600 TripleTOF™ System or 5800 MALDI-TOF/TOF™ System, see the ProteinPilot™ Software Help system. In addition, PDF versions of technical and application notes are available at http://www.sciex.com

For Research Use Only. Not for use in diagnostic procedures.

IMPORTANT! For all chemicals in bold red type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

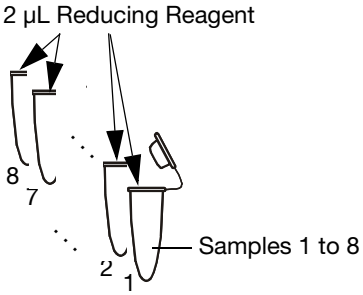
A Reduce the Proteins and Block Cysteine

1a. To each of up to eight sample tubes, add 20 µL Dissolution Buffer and 1 µL Denaturant.



b. Vortex to mix.

2a. To each sample tube, add 2 µL Reducing Reagent.

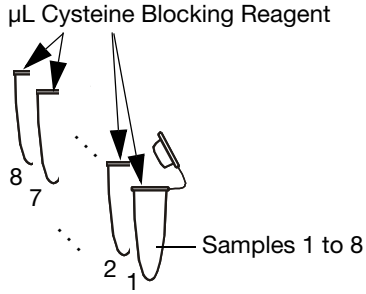


b. Vortex to mix, then spin.

c. Incubate the tubes at 60 °C for 1 hour.

d. Spin to bring the sample to the bottom of the tube.

3a. To each tube, add 1 µL Cysteine Blocking Reagent.

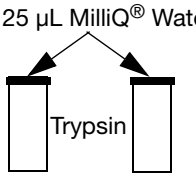


b. Vortex to mix, then spin.

c. Incubate the tubes at room temperature for 10 minutes.

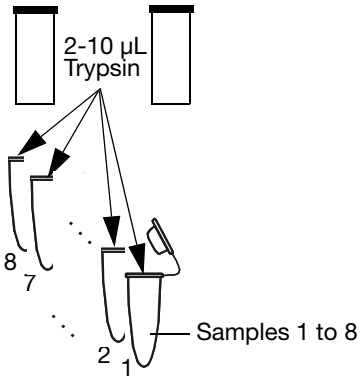
B Digest the Proteins with Trypsin

1a. For a 4plex experiment, reconstitute 1 to 2 vials of trypsin with 25 µL MilliQ® water or equivalent. (For an 8plex experiment, reconstitute 2 to 4 vials.)



b. Vortex to mix, then spin.

2a. To each sample tube, add 2 to 10 µL the trypsin solution.



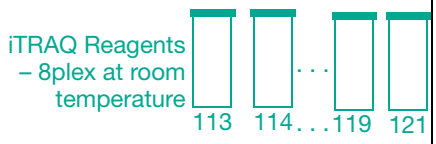
b. Vortex to mix, then spin.

c. Incubate at 37 °C overnight (12 to 16 hours).

d. Spin to bring the sample digest to the bottom of the tube.

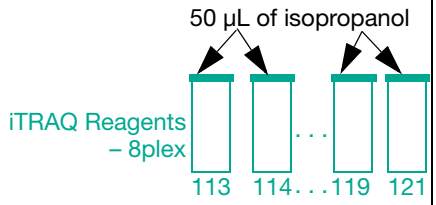
C Label Each Digest with an iTRAQ® Reagent – 8plex

1a. Allow iTRAQ® Reagents – 8plex to reach room temperature.



b. Spin to bring the solution to the bottom of the tube.

2a. To each iTRAQ Reagent – 8plex, add 50 µL of isopropanol.

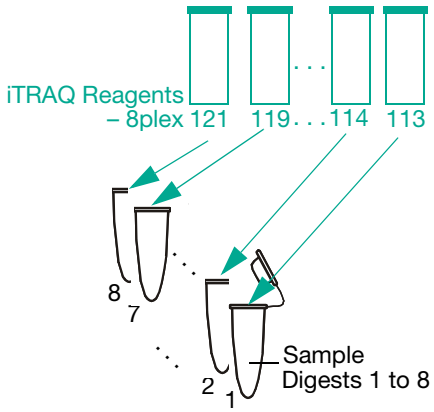


b. Vortex to mix, then spin.

(continued)

C Label (*continued*)

3a. Transfer the contents of one iTRAQ Reagent – 8plex vial to one sample tube.



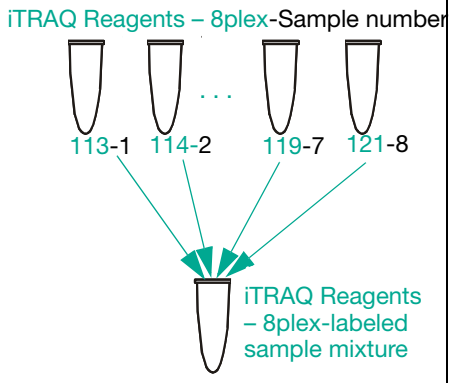
b. Vortex to mix, then spin.

c. If pH is <7.5, add up to 5 µL Dissolution Buffer.

d. Incubate at room temperature for 2 hours.

D Combine iTRAQ® Reagent – 8plex-Labeled Digests for Analysis

1a. Combine the contents of each iTRAQ Reagents – 8plex-labeled sample tube in a fresh tube.†



b. Vortex to mix, then spin.

IMPORTANT! Before LC/MS/MS analysis, clean up the sample mixture using cation exchange. If the sample mixture is complex, clean up and fractionate using high-resolution cation exchange.

†(Optional) Before combining the samples, reduce the organic solvent concentration, then clean up an aliquot of each labeled sample digest using a ZipTip®. Analyze each aliquot by MS/MS to verify that you see peaks at the m/z of the appropriate iTRAQ Reagents – 8plex reporter group. If not, relabel the protein digest.