Chemistry Quick Reference Card

This Quick Reference Card provides abbreviated procedures you can refer to when you use the iTRAQ® Reagents Multiplex Kit. For general chemical safety information, background information, a list of components required to perform the protocol, and detailed procedures, refer to the *xTRAQ Family of Amine -Modifying Labeling Reagents for Multiplexed Relative and Absolute Quantification: Chemistry Reference Guide* available at **http://www.sciex.com**).

Safety

CAUTION For safety guidelines and symbol identifications, refer to the "Safety Information" section in the *xTRAQ Family of Amine -Modifying Labeling Reagents for Multiplexed Relative and Absolute Quantification: Chemistry Reference Guide* and the Site Preparation Guide for your instrument. Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. Follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Cysteine Blocking Reagent is a flammable liquid and vapor. Exposure causes eye and respiratory tract irritation and central nervous system depression.
Denaturant (2% SDS) causes eye and skin irritation.
Ethanol is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage.
iTRAQ® Reagents114-117 cause eye and respiratory tract irritation. Exposure may cause blood damage.
Reducing Reagent causes eye, skin, and respiratory tract irritation.
Trypsin causes eye, skin, and respiratory tract irritation. Exposure may cause an allergic reaction.

Testing the Protocol

It is strongly recommended that, before running samples for the first time, you test the protocol as described in the *xTRAQ Family of Amine -Modifying Labeling Reagents for Multiplexed Relative and Absolute Quantification: Chemistry Reference Guide.* Materials for testing are in the iTRAQ® Reagents Methods Development Kit.

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, competing with peptide derivitization. If necessary, clean up the samples using acetone precipitation.

See the *xTRAQ Family of Amine -Modifying Labeling Reagents for Multiplexed Relative and Absolute Quantification: Chemistry Reference Guide,* for details on potentially interfering substances and sample clean up.

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.

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

Some substances (such as SDS, buffer salts, and high concentrations of organics) in an iTRAQ® Reagents-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-Labeled Sample Mixture

For information on analysis using the following AB SCIEX instruments and software see the product specific user guide which can be found at www.absciex.com:

- AB SCIEX 5600 TripleTOF™ System
- AB SCIEX QTRAP® System.
- AB SCIEX 5800 MALDI TOF/TOF™ Instrument
- AB SCIEX ProteinPilot™ Software

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

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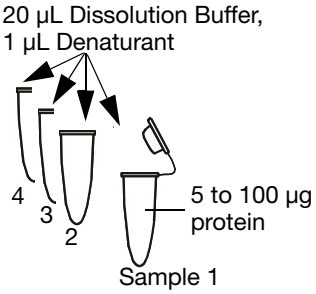
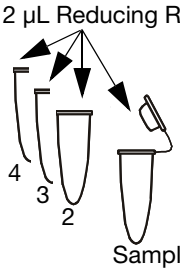
A Reduce the Proteins and Block Cysteine

1a. To each of up to four 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.

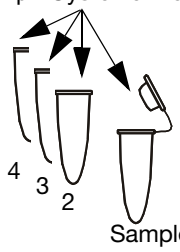


b. Vortex to mix, then spin.

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.

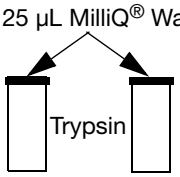


b. Vortex to mix, then spin.

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

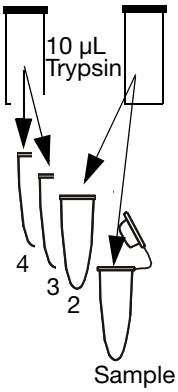
B Digest the Proteins with Trypsin

1a. Reconstitute a vial of trypsin with 25 µL MilliQ® Water or equivalent. (For three or four samples, reconstitute two vials.)



b. Vortex to mix, then spin.

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



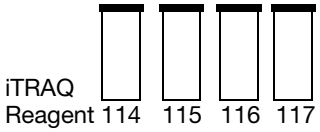
b. Vortex to mix, then spin.

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

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

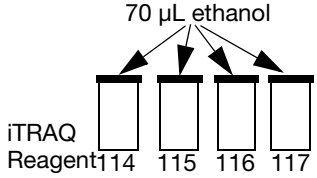
C Label the Protein Digests with iTRAQ® Reagents

1a. Allow iTRAQ™ reagent(s) required to reach room temperature.



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

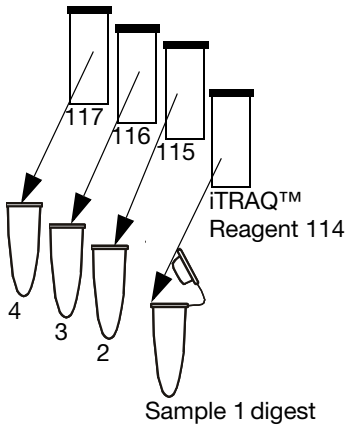
2a. To each iTRAQ™ reagent required, add 70 µL of ethanol.



b. Vortex to mix, then spin.

C Label the Protein Digests with iTRAQ® Reagents (*continued*)

3a. Transfer the contents of one iTRAQ® Reagent vial to one sample tube.

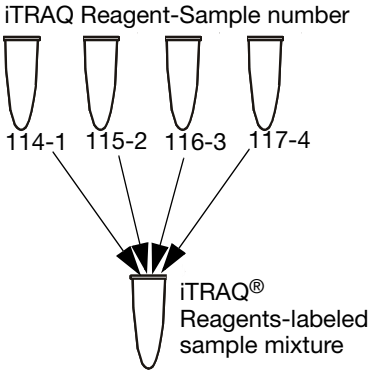


b. Vortex to mix, then spin.

c. Incubate at room temperature for 1 hour.

D Combining the iTRAQ® Reagent-Labeled Digests for Analysis

1a. Combine the contents of each iTRAQ® Reagent-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 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® Reagent reporter group. If not, relabel the protein digest.

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