mTRAQ® Reagents

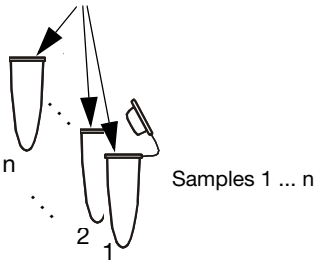
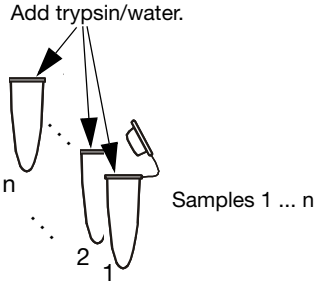
Amine-Modifying Labeling Reagents for Relative and Absolute Quantitation

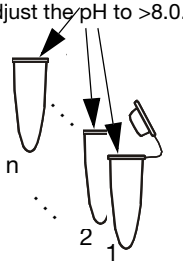
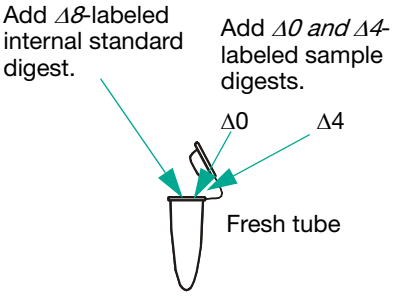
For safety and biohazard guidelines, refer to the "Safety" section in the *mTRAQ® Reagents Protocol* (PN 4373841). For all chemicals in bold red type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference card contains abbreviated procedures for labeling the internal standard, control, and test samples with mTRAQ® reagents. Refer to the *mTRAQ® Reagents Protocol* for complete procedures.

Perform the Labeling Protocol

IMPORTANT! The procedure below is written for a 1-unit vial of mTRAQ® Reagent. If you are using a 50-unit vial of mTRAQ® Reagent, you must aliquot and store the reagent properly to avoid hydrolytic degradation. Immediately after opening the vial, aliquot the appropriate volume (see the certificate of analysis) required to label samples into single-use tubes and store them under inert gas at –20 °C.

STEP	ACTION	
1	Reduce the proteins and block cysteines. Add Dissolution Buffer, Denaturant, Reducing Reagent, and Cysteine Blocking Reagent.  	a. To each sample tube containing 100 µg protein, add 20 µL Dissolution Buffer. b. Add 1 µL Denaturant, then vortex to mix. c. Add 2 µL Reducing Reagent, then vortex to mix and pulse-spin. d. Incubate the tubes at 60 °C for 1 hour. e. Pulse-spin to bring the material to the bottom of the tube. f. To each tube, add 1 µL Cysteine Blocking Reagent. Vortex to mix, then pulse-spin. g. Incubate the tubes at room temperature for 10 minutes.
2	Digest the proteins with trypsin. Add trypsin/water.  Incubate at 37 °C overnight (12 to 16 hours).	a. Reconstitute the vial(s) of trypsin with 25 µL Milli-Q® water. Vortex to mix, then pulse-spin. b. To each sample tube, add 10 µL of the trypsin solution. Vortex to mix, then pulse-spin. c. Incubate at 37 °C overnight (12 to 16 hours). d. Pulse-spin to bring the material to the bottom of the tube.

STEP	ACTION	
3	Label the internal standard digest(s) with mTRAQ Reagent Δ8. Add mTRAQ Reagent Δ8 isopropanol. Adjust the pH to >8.0.  Incubate at room temperature for 1 hour.	a. Bring the 1-unit vial(s) of mTRAQ Reagent Δ8 to room temperature. b. Pulse-spin to bring the material to the bottom of the tube. c. Add 50 µL of isopropanol. d. Vortex to mix, then pulse-spin. e. Transfer the contents of one mTRAQ Reagent Δ8 vial to each standard digest. f. Vortex to mix, then pulse-spin. g. If pH is <8.0, add up to 5 µL Dissolution Buffer. h. Incubate at room temperature for 1 hour.
4	Label each sample digest with mTRAQ Reagent Δ0 or Δ4. Add mTRAQ Reagent Δ0 to sample digest 1 and Δ4 isopropanol to sample digest 2. Adjust the pH to >8.0.  Incubate at room temperature for 1 hour.	a. Bring the 1-unit vial(s) of mTRAQ Reagent Δ0 and Δ4 to room temperature. b. Pulse-spin to bring the solution to the bottom of the tube. c. Add 50 µL of isopropanol. d. Vortex to mix, then pulse-spin. e. Transfer the contents of one mTRAQ Reagent Δ0 or Δ4 vial to sample digest. f. Vortex to mix, then pulse-spin. g. If pH is <8.0, add up to 5 µL Dissolution Buffer. h. Incubate at room temperature for 1 hour.
5	Create the analytical mixture for each control and test sample.  	a. For each labeled sample digest, combine equivalent aliquots of the mTRAQ Reagent Δ8-labeled internal standard and the mTRAQ Reagent Δ0 and Δ4-labeled sample digests in a fresh tube. b. Vortex to mix, then pulse-spin. c. Immediately clean up and analyze the analytical mixtures or store them at –20 °C. Refer to the *mTRAQ® Reagents Protocol*, Chapter 5, for cleaning up the mixtures using a cation-exchange procedure.

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