Chemistry Quick Reference Card

Note: For safety and biohazard guidelines, refer to the "Safety" section in the Amino Acid Analysis for Physiological Samples aTRAQ™ Reagents Application Kit Protocol (PN 4445547). For every chemical, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Amino Acids

A vial of AA Internal Standard contains approximately 9.0 nmole of each of the following amino acids labeled with aTRAQ™ Reagent Δ0:

- O-phospho-L-serine
- O-phospho-ethanolamine
- Taurine
- L-asparagine
- L-serine
- Hydroxy-L-proline
- Glycine
- L-glutamine
- Ethanolamine
- L-aspartic acid
- L-citrulline
- Sarcosine
- β-alanine
- L-alanine
- L-threonine
- L-glutamic acid
- L-histidine
- 3-methyl-L-histidine
- 1-methyl-L-histidine
- L-homocitrulline
- Argininosuccinic acid
- γ-amino-n-butyric acid
- D,L-β-amino-isobutyric acid
- L-α-amino-n-butyric acid
- L-α-aminoadipic acid
- L-anserine
- L-carnosine
- L-proline
- L-arginine
- δ-hydroxylysine
- L-ornithine
- Cystathionine
- L-cystine
- L-lysine
- L-valine
- L-norvaline
- L-methionine
- L-tyrosine
- L-homocysteine
- L-isoleucine
- L-leucine
- L-norleucine
- L-phenylalanine
- L-tryptophan

L-norvaline and L-norleucine are added during the precipitation and dilution steps of the protocol and are subsequently labeled with aTRAQ™ Reagent Δ8.

Allo-Isoleucine is provided as a separate standard that remains unlabeled.

Testing the Protocol

IMPORTANT! If you are running the protocol for the first time, it is strongly recommended that you practice performing the protocol to label the vial of AA Unlabeled Standard. For information, see the Amino Acid Analysis for Physiological Samples Protocol, Appendix C.

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.

Immediately before use:

- Briefly centrifuge the reagent and aTRAQ™ Reagent vials to dislodge material potentially trapped in the caps.
- Allow the reagents and each required vial of aTRAQ™ Reagent Δ8 to reach room temperature. Return the reagents to storage at -15 °C or below within 2 hours of thawing.
- Inspect the vial of Labeling Buffer. If precipitate is present, warm the vial to 37 °C, then vortex.

Analyzing the aTRAQ™ Reagent Δ8-Labeled Samples Using LC/MS/MS Analysis

For information on LC/MS/MS analysis, refer to the Amino Acid Analysis for Physiological Samples Protocol, Chapter 3.

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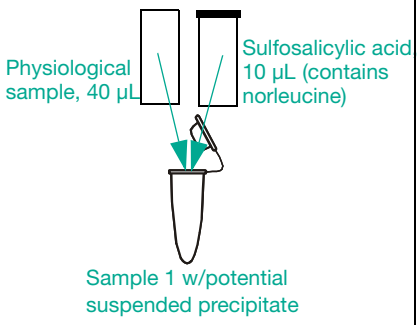
A Precipitate Sample Protein and Dilute

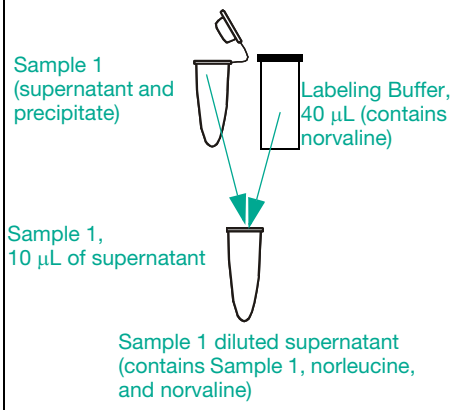
IMPORTANT! Always perform protein precipitation and dilution to incorporate norleucine and norvaline.

1a. Combine 40 µL of physiological sample and 10 µL of Sulfosalicylic acid.



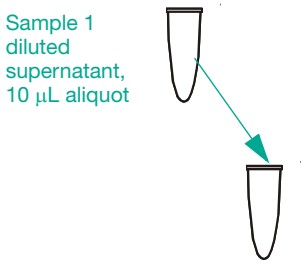
b. Vortex to mix, then spin at 10,000 ∞ g for 2 minutes.

2a. Transfer 10 µL of the supernatant to a clean tube, then add 40 µL of Labeling Buffer.



b. Vortex to mix, then spin. Save the sample to repeat labeling or to perform the optional allo-Isoleucine analysis (see step B4).

3. Transfer 10 µL of the diluted supernatant to a clean tube.

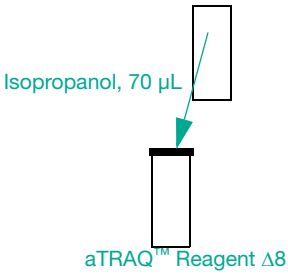


B Label the Samples with aTRAQ™ Reagent Δ8

1a. Allow a vial of aTRAQ™ Reagent Δ8 to reach room temperature (labels up to 15 assays).

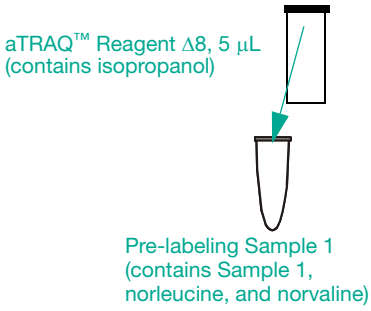
b. Spin to bring the solution to the bottom of the tube.

2a. Add 70 µL of Isopropanol.



b. Vortex to mix, then spin.

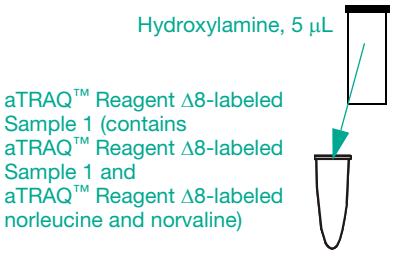
3a. To the Pre-labeling Sample (from step A3), add 5 µL of diluted aTRAQ™ Reagent Δ8. Store unused reagent at –15 °C or below.



b. Vortex to mix, then spin.

c. Incubate at room temperature for at least 30 min.

4a. Add 5 µL of Hydroxylamine.

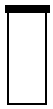


b. (Optional) For allo-Isoleucine analysis, add 5 µL of diluted supernatant from step A2.

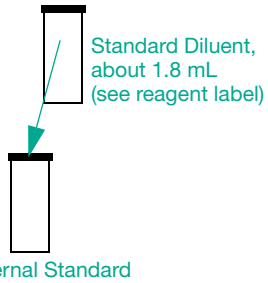
5. Dry the samples completely in a centrifugal vacuum concentrator (generally not more than 1 hour).

C Combine the aTRAQ™ Reagent Δ8-Labeled Sample and AA Internal Standard

1a. Spin a tube of AA Internal Standard to bring the reagent to the bottom of the tube.

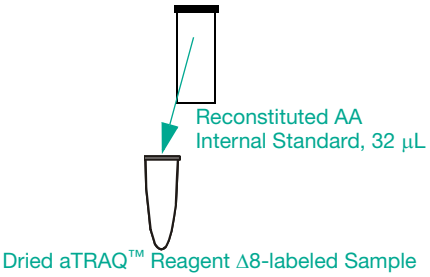


2a. Reconstitute a vial of AA Internal Standard with approximately 1.8 mL Standard Diluent (precise amount is indicated on the vial label).



b. Vortex in 30 to 60 sec increments until no precipitate is visible.

3a. Add 32 µL of reconstituted AA Internal Standard solution to each dried aTRAQ™ Reagent Δ8-labeled sample. Store unused standard at –15 °C or below.



b. Vortex to mix, then spin.

A 2-µL aliquot of the aTRAQ™ Reagent Δ8-labeled Sample and Reconstituted AA Internal Standard mix contains:

- aTRAQ™ Reagent Δ8-labeled amino acids in the sample
- 10 pmole of aTRAQ™ Reagent Δ8-labeled norvaline and norleucine
- Approximately 10 pmole of each Δ0-labeled amino acid from the AA Internal Standard, including norvaline and norleucine