

Chemistry Quick Reference Card

Note: For safety and biohazard guidelines, refer to the “Safety” section in the Amino Acid Analysis for Hydrolysate Samples aTRAQ™ Reagents Application Kit Protocol (PN 4445541). 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:

- L-serine
- Glycine
- L-aspartic acid
- L-alanine
- L-threonine
- L-glutamic acid
- L-histidine
- L-proline
- L-arginine
- L-methionine sulfoxide
- L-cystine
- L-lysine
- L-valine
- L-norvaline
- L-methionine
- L-tyrosine
- L-isoleucine
- L-leucine
- L-norleucine
- L-phenylalanine

L-norvaline is added during the reconstitution step of the protocol and is subsequently labeled with aTRAQ™ Reagent Δ8. To monitor the recovery, L-norleucine can be added to the sample prior to hydrolysis.

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 Amino Acid Unlabeled Standard. For information, see the Amino Acid Analysis for Hydrolysate 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 *Life Technologies Corporation Amino Acid Analysis for Hydrolysate Samples Protocol*, Chapter 3.

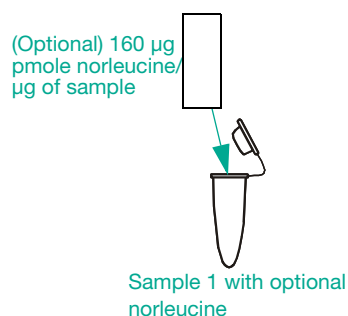
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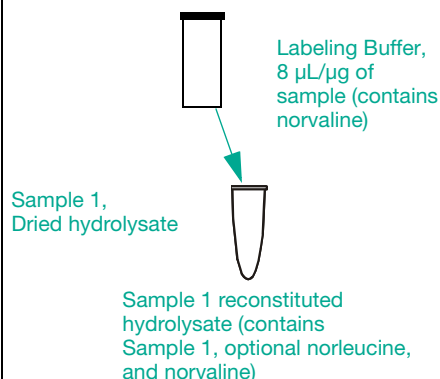
A Hydrolyze and Reconstitute the Sample

- 1a. Hydrolyze ≥ 1 μg of sample.
(Optional) Add 160 pmole of norleucine/ μg of sample.



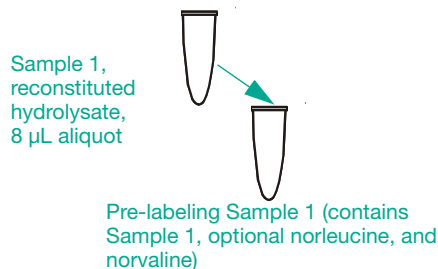
- b. Dry the hydrolyzed sample completely.

- 2a. Add 8 μL of Labeling Buffer for each μg of sample.



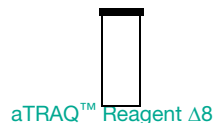
- b. Vortex to mix, then spin.

3. If >8 μL of Labeling Buffer was used in step 2, then transfer 8 μL of the reconstituted hydrolysate to a clean tube; otherwise, use the same tube from step 2.



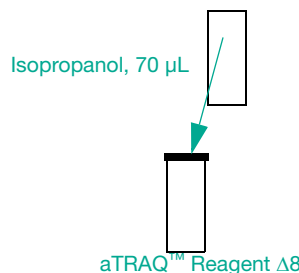
B Label the Samples with aTRAQ™ Reagent $\Delta 8$

- 1a. Allow a vial of aTRAQ™ Reagent $\Delta 8$ to reach room temperature (labels up to 15 assays).



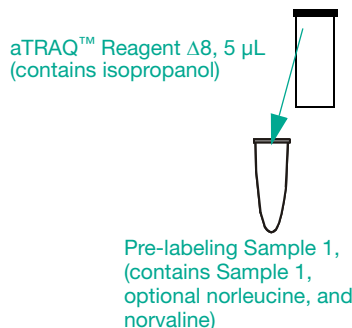
- b. Spin to bring the solution to the bottom of the tube.

- 2a. Add 70 μL of Isopropanol.



- b. Vortex to mix, then spin.

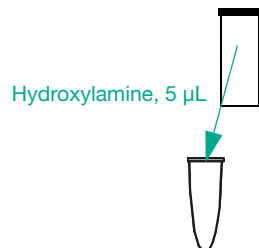
- 3a. To the Pre-labeling Sample (from step A3), add 5 μL of diluted aTRAQ™ Reagent $\Delta 8$. Store unused reagent at -15 $^{\circ}\text{C}$ or below.



- b. Vortex to mix, then spin.

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

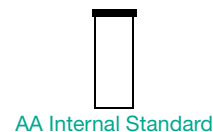
4. Add 5 μL of Hydroxylamine.



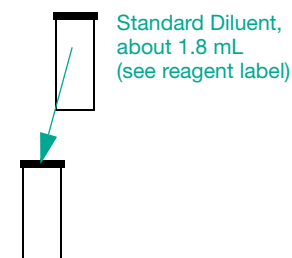
5. Dry the samples completely in a centrifugal vacuum concentrator (generally not more than 1 hour).

C Combine the aTRAQ™ Reagent $\Delta 8$ -Labeled Sample and AA Internal Standard

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

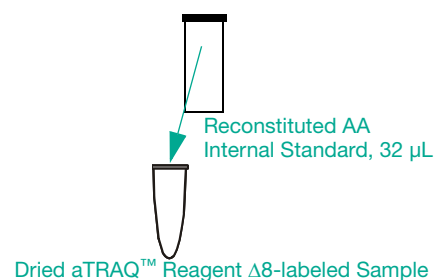


- 2a. Reconstitute a vial of AA Internal Standard with approximately 1.8 mL Standard Diluent (precise amount is indicated on the vial label).



- 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 $\Delta 8$ -labeled sample. Store unused standard at -15 $^{\circ}\text{C}$ or below.



- b. Vortex to mix, then spin.

A 2- μL aliquot of the aTRAQ™ Reagent $\Delta 8$ -labeled Sample and Reconstituted AA Internal Standard mix contains:

- aTRAQ™ Reagent $\Delta 8$ -labeled amino acids in the sample
- 10 pmole of aTRAQ™ Reagent $\Delta 8$ -labeled norvaline and optional norleucine
- Approximately 10 pmole of each $\Delta 0$ -labeled amino acid from the AA Internal Standard, including norvaline and norleucine