

# Chemistry Quick Reference Card

This Quick Reference Card provides abbreviated procedures you can refer to when you use the *Amino Acid Analysis for Physiological Samples Protocol* (available at <http://www.sciex.com>).

## Safety

For safety and biohazard guidelines, refer to the “Safety” section in the *Amino Acid Analysis for Physiological Samples Protocol*. For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Amino Acids

A vial of AA 45/32<sup>TM</sup> Phys Standard - 114 Labeled contains approximately 9.0 nmol of each of the following amino acids labeled with iTRAQ<sup>®</sup> Reagent 114:

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

L-norvaline and L-norleucine are added during the precipitation and dilution steps of the protocol and are subsequently labeled with iTRAQ Reagent 115.

Allo-isoleucine is provided as a separate standard that remains unlabeled and is analyzed using the allo-isoleucine method (see *Amino Acid Analysis for Physiological Samples Protocol*, **Appendix B**).

## 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 45/32 Standard - Unlabeled. 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 iTRAQ Reagent vials to dislodge material potentially trapped in the caps.
- Allow the reagents and each required vial of iTRAQ Reagent 115 to reach room temperature. Return the reagents to storage at -15 to -25 °C 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 iTRAQ<sup>®</sup> Reagent 115-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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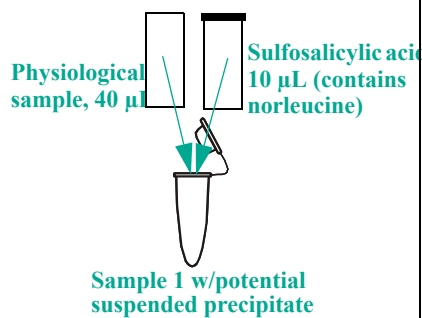
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## A Precipitate Sample Protein and Dilute

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

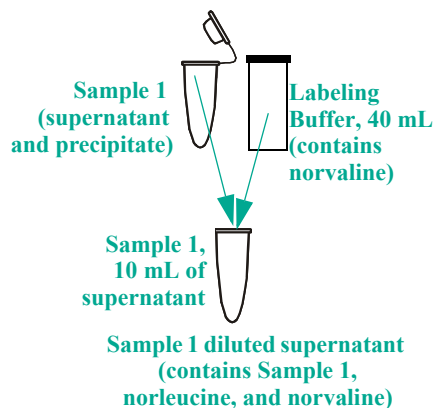
- 1a. Combine 40  $\mu\text{L}$  of **physiological sample** and 10  $\mu\text{L}$  of Sulfosalicylic acid.



- b. Vortex to mix, then spin at  $10,000 \times g$  for 2 minutes.

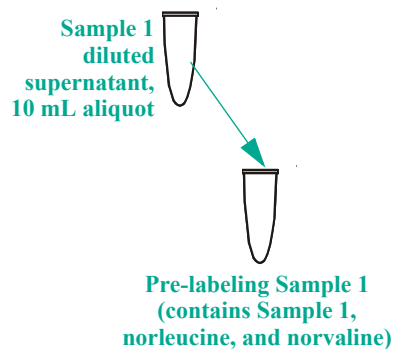
- 2a. Transfer 10  $\mu\text{L}$  of the supernatant to a clean tube, then add 40  $\mu\text{L}$  of Labeling Buffer.

(Optional) Transfer 20  $\mu\text{L}$  of supernatant to a clean auto-sampler vial for alloisoleucine analysis.



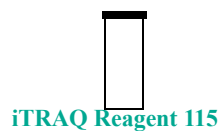
- b. Vortex to mix, then spin.

3. Transfer 10  $\mu\text{L}$  of the diluted supernatant to a clean tube.



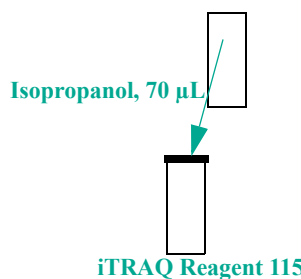
## B Label the Samples with iTRAQ® Reagent 115

- 1a. Allow a vial of **iTRAQ Reagent 115** to reach room temperature (labels up to 15 assays).



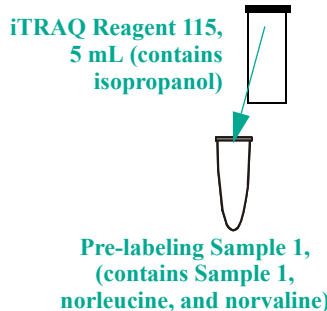
- b. Spin to bring the solution to the bottom of the tube.

- 2a. Add 70  $\mu\text{L}$  of **Isopropanol**.



- b. Vortex to mix, then spin.

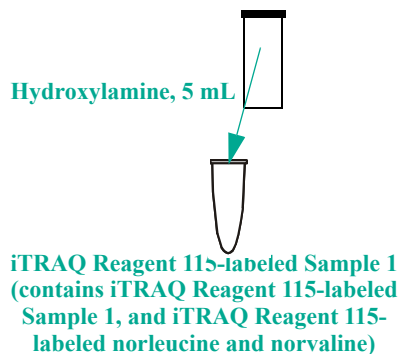
- 3a. To the Pre-labeling Sample (from step A1), add 5  $\mu\text{L}$  of diluted iTRAQ Reagent 115. Store unused reagent at  $-15$  to  $-25$   $^{\circ}\text{C}$ .



- b. Vortex to mix, then spin.

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

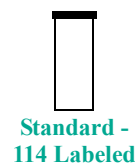
- 4a. Add 5  $\mu\text{L}$  of Hydroxylamine.



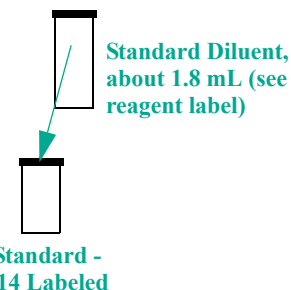
5. Dry the samples completely in a centrifugal vacuum concentrator (generally not more than 1 hour).

## C Combine the iTRAQ® Reagent 115-Labeled Sample and iTRAQ® Reagent 114-Labeled Standard

- 1a. Spin a tube of **Standard - 114 Labeled** to bring the reagent to the bottom of the tube.

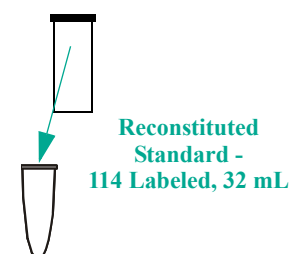


- 2a. Reconstitute a vial of Standard - 114 Labeled with approximately 1.8 mL Standard Diluent (precise amount is indicated on the vial label).



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

- 3a. Add 32  $\mu\text{L}$  of reconstituted Standard - 114 Labeled to each dried iTRAQ Reagent 115-labeled sample. Store unused standard at  $-15$  to  $-25$   $^{\circ}\text{C}$ .



**Dried iTRAQ Reagent 115-labeled Sample**

- b. Vortex to mix, then spin.

A 2- $\mu\text{L}$  aliquot of the iTRAQ Reagent 115-labeled Sample and Reconstituted Standard - 114 Labeled mix contains:

- iTRAQ Reagent 115-labeled amino acids in the sample
- 10 pmol of iTRAQ Reagent 115-labeled norvaline and norleucine
- 10 pmol of each iTRAQ Reagent 114-labeled amino acid in the standard, including norvaline and norleucine