Amino Acid Analysis Quick Reference Card

For Hydrolysate Samples

Safety

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

Overview

This document supports amino acid analysis for hydrolysate samples using iTRAQ™ Reagents and the AB SCIEX Amino Acid 20/20 Analyzer.

The labeling protocol labels a peptide hydrolysate, protein hydrolysate, or a hydrolysate from animal feed sample (dry ≈ 10 nmol amino acid) with iTRAQ Reagent 117. An iTRAQ™ Reagent 114-labeled amino acid standard is added as an internal standard.

See the Amino Acid Analysis for Hydrolysate Samples Protocol for:

- Pipetting recommendations.
- Recommended analysis conditions, quality control, and troubleshooting.
- Supplemental information such as "How to Obtain More Information."

Amino Acid Analysis of Hydrolysate Samples - iTRAQ™ Reagents Labeling Protocol

IMPORTANT! Throughout the procedure, cap each tube promptly to avoid evaporation.

Labeling the Hydrolysate Sample with iTRAQ Reagent 117

1. If necessary, dry the hydrolysate sample.

   IMPORTANT! For optimal labeling, the hydrolysate sample must be completely dry.

2. Add Labeling Buffer - Amino Acid†:
   - To each sample tube containing 1 µg hydrolysate:
     a. Add 5 µL Labeling Buffer - Amino Acid.
     b. Vortex to mix, then spin.
   - To each sample tube containing more than 1 µg hydrolysate:
     a. For every 1 µg of hydrolysate, add 5 µL of Labeling Buffer - Amino Acid. For example, if your sample contains 6 µg of hydrolysate, add 30 µL of Labeling Buffer - Amino Acid
     b. Vortex to mix, then spin.
3. Spin each required vial of iTRAQ Reagent 117 (at room temperature) to bring the solution to the bottom of the vial.
4. Add 70 µL of isopropanol. Mark the vial as "diluted."
5. Vortex each vial to mix, then spin.
6. Label each hydrolysate sample prepared in step 2 with iTRAQ Reagent 117:
   - To each sample tube containing 1 µg hydrolysate:
     a. Add 5 µL of diluted iTRAQ Reagent 117.
     b. Vortex to mix, then spin.
   - To each sample tube containing more than 1 µg hydrolysate:
     a. Transfer a 5-µL aliquot of the hydrolysate sample/Labeling Buffer - Amino Acid solution to a fresh tube.
     b. To the aliquot, add 5 µL of diluted iTRAQ Reagent 117.
     c. Vortex to mix, then spin.
7. Incubate the sample tubes at room temperature for at least 30 min.
8. Add 1 µL of hydroxylamine to each sample tube.
9. Vortex each sample tube to mix, then spin.
10. Incubate the sample tubes at room temperature for at least 5 min.
11. Dry the samples completely in a centrifugal vacuum concentrator (generally not more than 1 hour).

Adding iTRAQ Reagent 114-Labeled Internal Standard

1. Prepare a 6-pmol/µL iTRAQ™ Reagent 114-labeled amino acid internal standard by reconstituting one vial of Hydrolysates Standards - 114 Labeled with Sample Diluent - Amino Acid. The amount of Sample Diluent - Amino Acid to use is indicated on the Certificate of Analysis (approximately 1.67 mL).
2. Vortex to mix, then spin.

   The iTRAQ™ Reagent 114-labeled amino acid internal standard can be stored at –15 to –25 °C.
3. Add 25 µL of the iTRAQ™ Reagent 114-labeled amino acid internal standard to each dried iTRAQ Reagent 117-labeled sample.
4. Vortex each tube to mix, then spin.

IMPORTANT! This procedure yields enough material for approximately three 5-µL injections for each sample. Discard any remaining material.

iTRAQ™ Reagent-Labeled Amino Acids in a 5-µL Injection

A 5-µL injection prepared according to the protocol contains:

- iTRAQ Reagent 117-labeled amino acids in the sample
- 30 pmol of iTRAQ Reagent 117-labeled norvaline
- 30 pmol of each iTRAQ Reagent 114-labeled amino acid in the standard, including norvaline

† Labeling Buffer - Amino Acid contains 30 pmol/µL norvaline.

Equilibrating the Column Before Reuse

1. Wash the column with 2.5 mL 30% Mobile Phase A/ 70% Mobile Phase B at 1.0 mL/min for 2.5 min.
2. Equilibrate the column with 25 mL of 98% Mobile Phase A/ 2% Mobile Phase B (initial starting conditions) at 1.0 mL/min for 25 min.
3. To verify a stable baseline, continue the 98% Mobile Phase A/2% Mobile Phase B flow and inject 5 µL Sample Diluent - Amino Acid (3.5% formic acid) at least twice.

Mobile Phases, Gradient, and Chromatogram

Table 1 Recommended mobile phase preparation (1L)

Component	Mobile Phase A	Mobile Phase B
Mobile Phase Modifier A	1.00 mL	1.00 mL
Mobile Phase Modifier B	50.0 µL	50.0 µL
Milli-Q® water HPLC grade	998.95 mL	___
Acetonitrile, HPLC-grade	___	998.95 mL

Table 2 Recommended LC gradient

Total Time (min)‡	%Mobile Phase A	%Mobile Phase B
0.0	98.0	2.0
10.0	72.0	28.0
10.1	0.0	100.0
16.0	0.0	100.0
16.1	98.0	2.0
25.0	98.0	2.0

‡ Recommended flow rate: 1.0 mL/min, split in the source to 200 to 250 µL/min

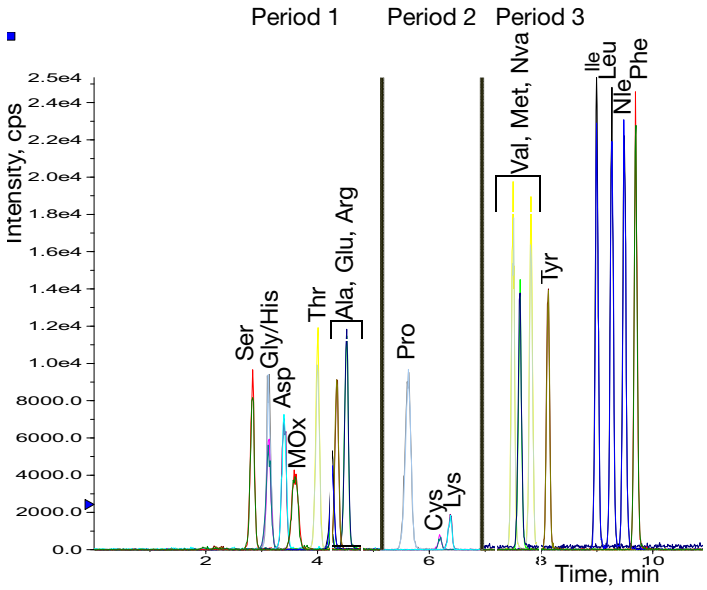

Figure 1 Representative chromatogram

Amino Acid Specifications

For each period in the MRM experiment:

- Q1 for each iTRAQ Reagent 114-labeled amino acid in the internal standard and the corresponding iTRAQ Reagent 117-labeled amino acid in the sample is the labeled mass shown in the table.
- Q3 for each labeled amino acid in the internal standard = 114.
- Q3 for each labeled amino acid in the sample = 117.

Name	Formula	MH+ (amu)	
		Unlabeled	Labeled (Q1)
Period 1, Experiment 1 Dwell = 50			
L-serine	$C_3H_7NO_3$	106.1	250.2
glycine	$C_2H_5NO_2$	76.0	220.1
L-histidine	$C_6H_9N_3O_2$	156.1	300.2
L-aspartic acid	$C_4H_7NO_4$	134.0	278.2
L-methionine sulfoxide	$C_5H_{11}NO_3S$	166.1	310.2
L-threonine	$C_4H_9NO_3$	120.1	264.2
L-alanine	$C_3H_7NO_2$	90.1	234.2
L-glutamic acid	$C_5H_9NO_4$	148.1	292.2
L-arginine	$C_6H_{14}N_4O_2$	175.1	319.2
Period 2, Experiment 1 Dwell = 100			
L-proline	$C_5H_9NO_2$	116.1	260.2
L-cysteine	$C_3H_7NO_2S$	122.0	266.1
L-lysine (2 labels)	$C_6H_{14}N_2O_2$	147.1	435.3
Period 3, Experiment 1 Dwell = 50			
L-valine	$C_5H_{11}NO_2$	118.1	262.2
L-norvaline	$C_5H_{11}NO_2$	118.1	262.2
L-methionine	$C_5H_{11}NO_2S$	150.1	294.2
L-tyrosine	$C_9H_{11}NO_3$	182.1	326.2
L-isoleucine	$C_6H_{13}NO_2$	132.1	276.2
L-leucine	$C_6H_{13}NO_2$	132.1	276.2
L-norleucine	$C_6H_{13}NO_2$	132.1	276.2
L-phenylalanine	$C_9H_{11}NO_2$	166.1	310.2

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