# A Rapid iMethod™ Test for the Analysis of 45 Amino Acids in Physiological Fluids

***iMethod™ Test for Amino Acids Analysis with Cliquid® AA45 Software 3.0***

The following description outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the analysis of 45 Amino Acids in physiological fluid using amino reactive isotope coded tags in conjunction with LC/MS/MS. This method has been verified for use on both AB SCIEX 3200 and 4000 level LC/MS/MS systems.

This method is for the routine analysis of 45 Amino Acids in urine, serum and plasma samples. Included with this iMethod Test is Cliquid® AA45/20 Software 3.0. Please note that an HPLC column and either the AA45/32 or AA20/20 Starter Kit will need to be purchased separately.

The isomeric and isobaric amino acids are separated using unique chemistries. Calibration is performed using an internal standard for each amino acid to compensate for any instrument variability.

Sample preparation in this method is based upon a simple protein precipitation and derivitization with iTRAQ® Reagents. The total run time is less than 13 minutes.

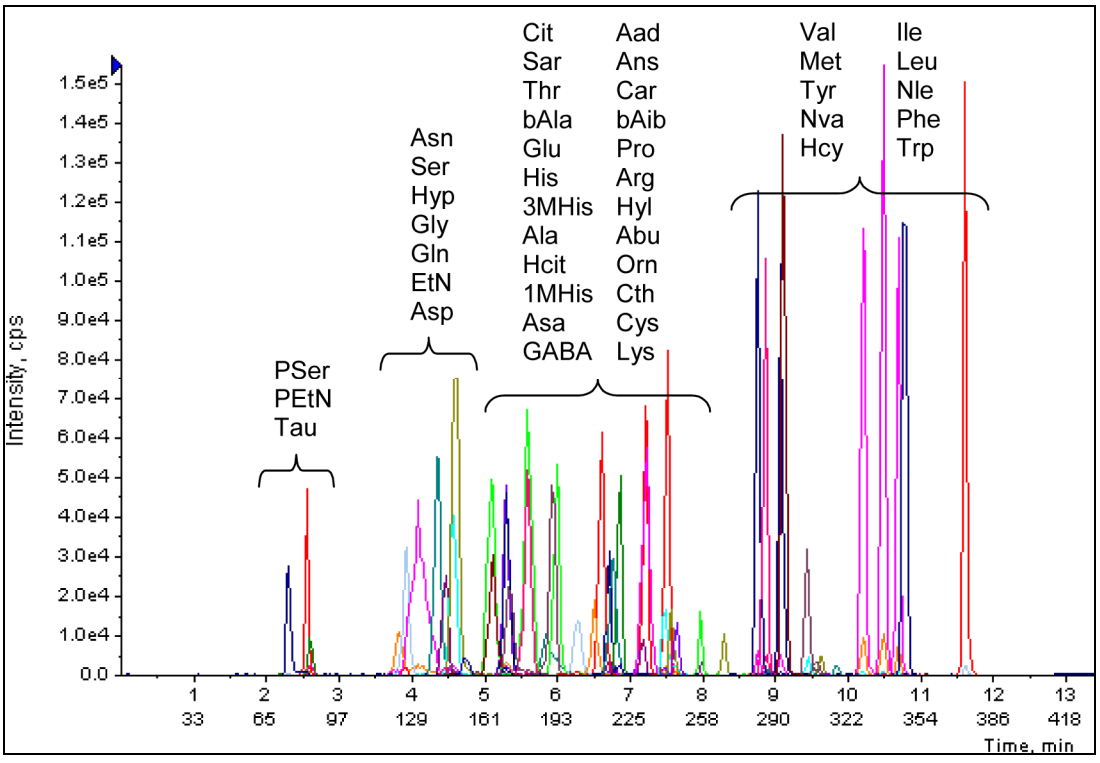


**Figure 1. Separation of 45 Amino Acids in less than 13 minutes**

## Calibration

Calibration curves were constructed using the controls in the AA45/32 Starter Kit with limits of quantitation ranging from 0.5 to 10 µM with a dynamic range of 3 to 4.5 orders of magnitude.

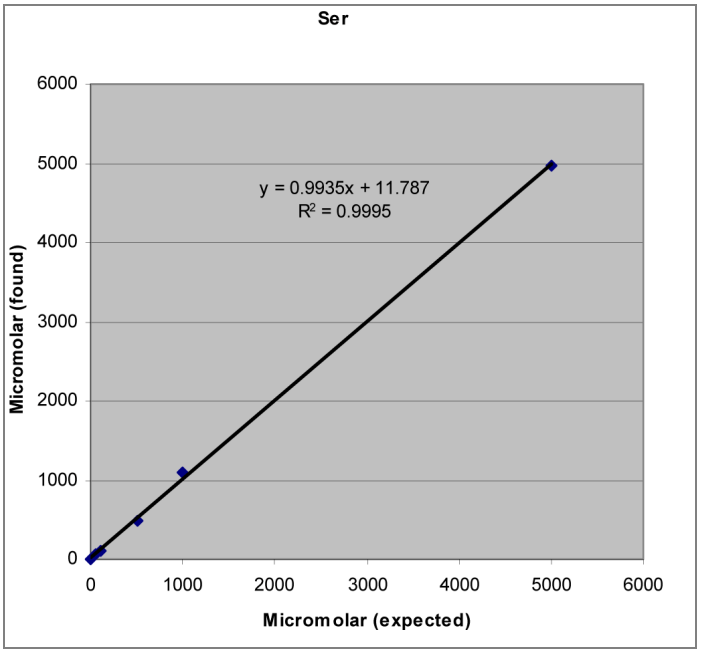


**Figure 2. Representative calibration curves for Serine.**

Please note that the results presented above were obtained using a single instrument and single set of standards and samples. Prior to production use, the method should be fully validated with real samples, and the results here may not be typical for all instruments. Variations in LC column properties, chemicals, environment, instrument performance and sample preparation procedures will impact performance, thus these results should be considered as informative rather than representative.

## System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX 3200 Series (3200 QTRAP® or API 3200™) or 4000 Series (4000 QTRAP® or API 4000™) LC/MS/MS System
- An Agilent 1100/1200 LC system with binary pump G1312A (without static mixer), well plate auto sampler, and thermostated column oven.
- AA 45/32 Starter Kit, Part No# 4374891 (iTRAQ® Reagents, Internal Standards, Control Plasma, Users Guide)
- An AB SCIEX AAA LC Column, Part No# 4374841
- LC/MS Grade water, methanol, hexane and acetonitrile
- Pipettes and standard laboratory glassware

## Ordering Information

| Product Name | Part Number |
|---|---|
| *iMethod™ Test for Amino Acids Analysis with Cliquid® AA45 Software 3.0* | 1037778 |

While the information provided above outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the Analysis Amino Acids, please note that the results obtained do require some experience with LC/MS/MS and sample preparation procedures. As such, web-based and on-site training are available to assist in the deployment of the iMethod™ Test and are recommended for inexperienced users. Please consult your local sales representative for more details.

## Important Note

The iMethod™ Test described above has been designed by AB SCIEX to provide the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. This method is provided for information purposes only. The performance of this method is not guaranteed due to many different potential variations, including instrument performance, tuning, and maintenance, chemical variability and procedures used, technical experience, sample matrices, and environmental conditions. It us up to the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

The purchase and use of certain of the chemicals listed below may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed below are for illustrative purposes only and may or may not meet the aforementioned local requirements. AB SCIEX is not responsible for user's compliance with any statute or regulation, or for any permit or approval required for user to implement any iMethod™ procedure.

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**Headquarters**
353 Hatch Drive | Foster City CA 94404 USA
Phone 650-638-5800
www.absciex.com

**International Locations**
For our office locations please call the division headquarters or refer to our website at
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