

FOR IDENTIFICATION AND COMPARABILITY OF 100S OF METABOLITES ACROSS TIME AND INSTRUMENTS

Background

LC-MS/MS metabolomics methods use isotopically labeled internal standards to ensure reproducibility and accuracy as they behave physically and chemically identical to the analytes under measurement.

To ensure reproducibility and accuracy of measurements, IROA Technologies developed the IROA WorkFlow for use in the analysis of any type of research or clinical sample.

Benefits of the IROA Workflow (see opposite side)

Cost effective identification and measurement of 100's of analytes using unique complex stable-labeled (U- 95% ¹³C) IROA Internal Standard (**IROA-IS**)

- Native and labeled metabolites easily distinguished
- Co-location of native compounds in experimental samples, even at low concentrations
- Native and labeled metabolites chromatographically separate together and ionize with the same intensity
- Fragments and adducts have identical IROA labeling patterns of precursor ions and can be identified
- Artfactual or non-paired peaks can be eliminated
- IROA-IS generated Retention Time (RT) ladder allows alignment of all peaks
- Metabolite data may can be normalized to the IROA-IS to overcome day-to-day, instrument-to-instrument variances

IROA-Matrix (paired mixture of IROA-IS and its U-5% ¹³C mirror image)

- Used to build a reference **library of compounds** for each method or LC-MS mode
- Analyzed every 10 samples to create a Retention Index specific to the chromatographic run each day
- Enables comparison of data across time and instruments

ClusterFinder Software

- Developed to automatically find, quantitate and identify all natural abundance peaks in experimental samples corresponding to known IROA isotopomers in the IROA-IS and remove artifacts
- Generates libraries for each LC-MS mode using data from Matrix analysis
- Workflow ensures **high level QC** for accurate and reproducible results

IROA WORKFLOW



Suggested Use

Injection Standard

Recovery Standard

Build libraries for each LC-MS Mode

Targeted/untargeted analyses
(Research or Clinical Samples)

IROA-WORKFLOW Kit includes

materials and tools for the analysis of 90 experimental samples

Unique fully-labeled Yeast Extract

- 3 vials of lyophilized IROA-IS
- 3 vials of lyophilized IROA-Matrix
- **ClusterFinder™ software**
- User manual

Store at -80°C

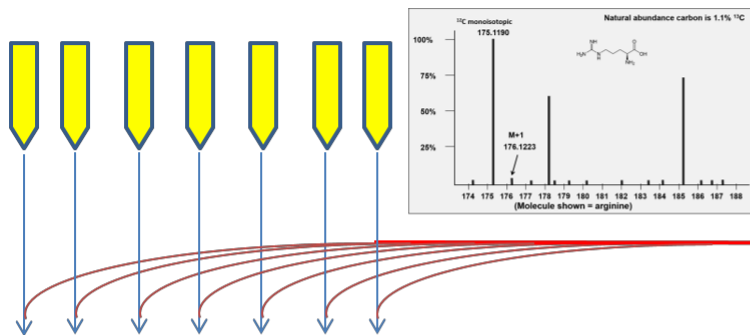
FOR RESEARCH USE ONLY

Proprietary IROA-labeled materials specially produced for IROA Technologies by Cambridge Isotope Laboratories (CIL).

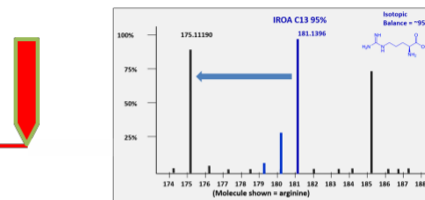
IROA WORKFLOW



Experimental Samples



+ Internal Standard (IS)



Experimental Samples + IS



Experimental Samples + IS



Experimental Samples + IS



Matrix

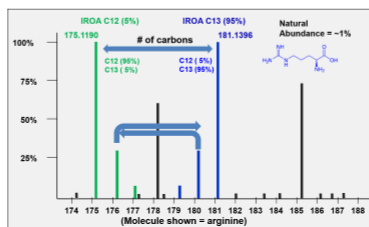


Figure. The IROA-based workflow adds a consistent biochemically complex Internal Standard into every experimental sample for enhanced quantitation, and two injections of a specially-developed, pure IROA matrix sample are analyzed every 10 samples to support identification, compound location and to create a Retention Index specific to the chromatographic run each day. Together these assure the comparability of accurate data across time and instruments.