IROA TruQuant IQQ WORKFLOW KIT

Catalog Number **IROA-WORKFLOW**

Identification, quantitation and QC of metabolites across time and instruments

IQQ

Identification: **Confidence in identifying common and new metabolites**
Quantification: **Comprehensive correction of analytical and biological variability for all analytes**
Quality Control: **Convenient and automated tools for assuring reproducibility within/across studies**

Background

IROA TruQuant/IQQ is a high-quality quantitation system that utilizes (2) complex Internal Standards to make accurate biological measurements on several hundred biochemicals simultaneously in small quantities of biological samples.

(1) A spiked in Internal Standard (IROA-IS) is used to: a) quantify 100's of biochemicals in a sample; b) suppression-correct each compound and c) normalize sample to sample variances. (2) A Long-Term Reference Standard (IROA-LTRS) is analyzed every 10 samples to create a daily RT library of all compounds to be found in the IROA-IS for reproducible ID and to measure day-to-day (QA/QC) to assure reproducible instrument performance.

The system is completely automated using IROA ClusterFinder software.

3 STEP PROCESS: (see Figure opposite side)

(1) LC-MS analysis of experimental samples spiked with **IROA-IS**. Inject **IROA-LTRS** periodically i.e. 10 sample intervals, as QC Standard to account for fluctuations in mass and RT drift.

(2) Generate "dictionary" of RT, m/z, formula and physical characteristics from the analysis of IROA-LTRS using ClusterFinder software.

(3) ClusterFinder and dictionary identifies, quantitates, suppression-corrects and normalizes compounds in experimental samples.

IROA LONG-TERM REFERENCE STANDARD – IROA-LTRS (paired 1:1 mixture of labeled U-5% and - 95% U-13C metabolites): **ID and QC** - used to build a triply redundant dictionary (library) of RT, *m/z* and physical characteristics including fragmentation data and to account for fluctuations in mass and RT.

IROA INTERNAL STANDARD – IROA-IS (labeled 95% U-13C metabolites): - spiked into experimental samples and used as a yardstick in which to **QUANTIFY** all biochemicals in the sample relative to their counterparts in the IROA-IS. Even if analyzed on a different chromatographic system, results can be equated using dictionary because of IROA-IS. Experimental compounds may have different RTs but will exhibit the same mass and physical characteristics.

Summary of Benefits of the IROA TruQuant (IQQ) Measurement System

- **Cost effective** simultaneous metabolite identification, quantitation and platform QC
- Accurate compound formula ID for MS alone; complete ID with addition of SWATH, or IM
- **IROA patterns discriminate peaks of biological origin from artefactual peaks** allowing the removal of false data
- IROA-based **fragments** have the IROA ratio pattern of their parent peaks and can be **identified** as such using the "peak correlation" ClusterFinder module
- Retention Index within the sample **allows "unknowns" to be tracked** with precision
- **Suppression-corrected** measurements for significantly better quantitation
- **Normalizes** (suppression-corrected) experimental samples to overcome day-to-day, instrument-to-instrument variances
- **ClusterFinder software solution** builds libraries, IDs/quantitates compounds and normalizes data
- Ensures **high level QC** for accurate and **reproducible** results

IROA TruQuant IQQ Workflow

QC MEASUREMENT SYSTEM for targeted or untargeted analyses

Suggested Use

Platform Quality Control, method optimization and benchmarking

Research and clinical sample ID, quantification, suppression-correction and normalization

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-LTRS**
- **ClusterFinder™ software (CF)**
- **Library of all IROA peaks in the LTRS and their physical characteristics** (provided in CF)
- User manual

Store at -20°C, long-term at -80°C, Protected from light
FOR RESEARCH USE ONLY

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

IROA WORKFLOW

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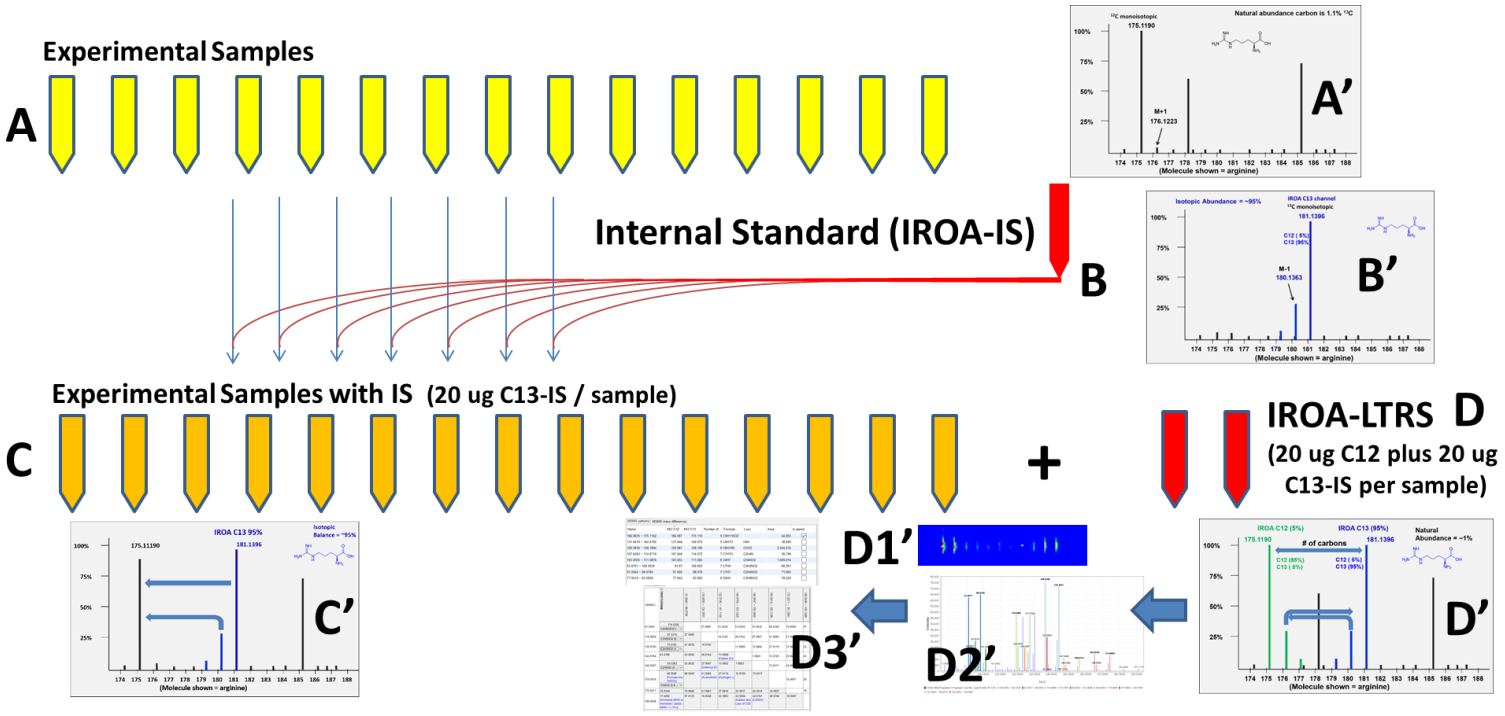

The IROA TruQuant IQQ workflow adds a consistent biochemically complex Internal Standard (B) into every experimental sample (A) for enhanced quantitation. Injections of a well-characterized Long-Term Reference Standard (D=LTRS) are analyzed every 10 samples to support identification, compound location and to create a Retention Index specific to the chromatographic run each day. Together these provide instrument and process QA/QC, validated compound identification and quantitation.

The IROA-LTRS "U-shaped smile" pattern of peaks (D') contains both the IROA-IS envelope (U-95% ^{13}C peaks; M-1 etc.) and its mirror-image envelope (U-5% ^{13}C peaks; M+1 etc.). The height of the M+1 and M-1 differ directly according to the number of carbons in a molecule; here 32% the height of their monoisotopic peaks, ^{12}C and ^{13}C, for a six-carbon molecule. This is true not only for the M+1 and M-1, but also the shape of the entire isotopic envelope is different for every number of carbons. The number of carbons in a biological molecule can be also determined by the distance between the two monoisotopic peaks. Since the relative height of the M+1, the relative height of M-1, and the distance between the monoisotopic peaks all provide confirmation of this fact, this results in a triply redundant quality control check point.

The IROA-LTRS is initially analyzed separately using LC-MS and MSMS methods to build a "dictionary" of RT, m/z and physical characteristics stored in ClusterFinder database. A comprehensive IROA-LTRS library is built from LC-MS analysis coupled with data collected detailing physical characteristics from added second-stage orthogonal analyses such as Ion Mobility or SWATH fragmentation. Information in the dictionary is subsequently used to ID metabolites in experimental samples spiked with the IROA-IS. The IS serves as a yardstick. Even if a different chromatographic system is used the software can rely on the physical characteristics stored in the dictionary to accurate ID compounds.

The IROA IM peaks retain their patterns perfectly because all IROA isotopomers share the same CCS (D'). In IROA msms fragmentation, such as SWATH, the IROA peaks retain their patterns (D2') because wide windows are used. Since all fragments retain their IROA character, their formulae (D3') and the relationships between them (D4') are determinable.