IROA BIOCHEMICAL QUANTITATION KITS

IROA® Background Overview

The IROA® Biochemcial Quantitation Kits are supplied with reagents and tools for the successful *in-vivo* labeling, quantitation and identification of biochemical compounds in many fungi, bacterial, and mammalian cell populations when combined with sample preparation and mass spectrometry (MS).

Unlike other labeling techniques which utilize "heavy" and "light" forms of isotopes, the IROA® protocol is based on labeling with carbon sources based on 95% and 5% U-^{13}C. This is done so that not only the monoisotopic peaks (usually the base peak) are detected during MS analysis, but also the carbon envelop of associated isotopic peaks can be detected. The important diagnostic information of these envelopes is central to the IROA protocol to provide meaningful and accurate data. The carbon envelop is used to: 1) differentiate control and experimental samples from each other and also from artifacts, 2) identify compounds of interest in the sample and calulate the number of carbons in each molecule, 3) reduce experimental error, and provide unambiguous and redundant quality control checks.

Provided with IROA kits: 1) Labeling media and components, 2) IROA ClusterFinder™ software tool, developed to characterize all peaks according to source (artifact, control, experimental), remove artifacts, quantitate and identify biochemicals, and; 3) access to the IROA Portal, which enables high quality data interpretation of an IROA dataset including basic and advanced statistical analyses, providing a total metabolomics solution. The user can also employ their favorite statistical package for any additional analysis.

The kits are designed to quantitate metabolic differences between groups of cell populations, a Control group and any number of Experimental groups. The Experimental group is typically either treated with a stimulus or stressor or genetically modified. The Basic IROA® workflow is illustrated below.

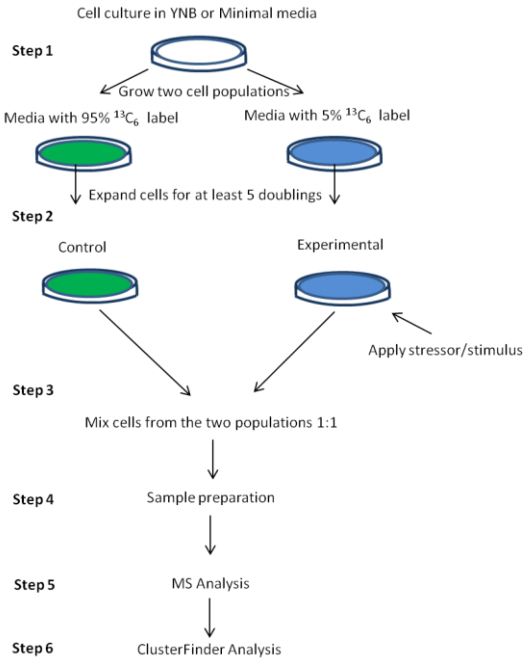
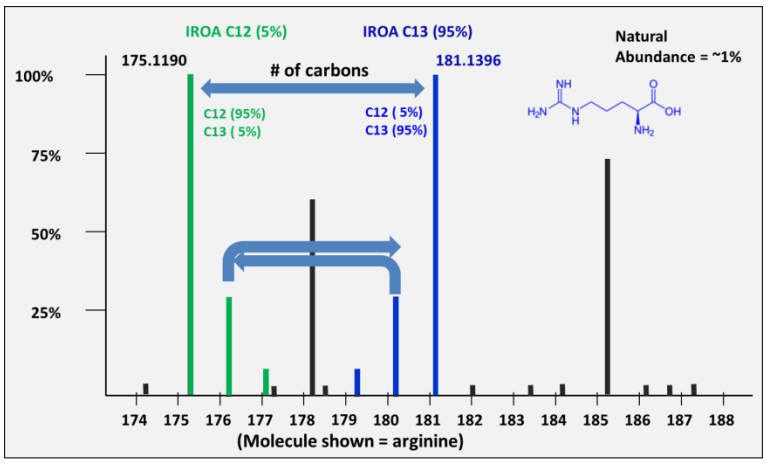
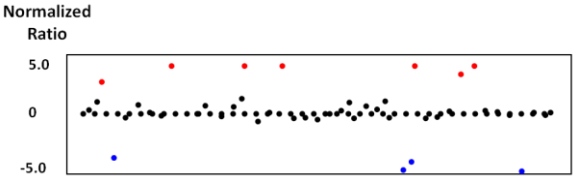


Figure: Basic IROA Work Flow

As each metabolite pair (Control and Experimental) is identified by the ClusterFinder software (arginine shown below), its ratio is calculated, normalized and stored. Outliers to the normalized ratios are compounds that were altered by the experimental condition.





IROA = Isotopic Ratio Outlier Analysis

Description of the IROA® Kits and Media

IROA (Isotopic Ratio Outlier Analysis) is used for comparative quantative metabolic profiling in fungal, bacterial and mammalian cultured cells.

IROA Biochemical Quantitation Kit Catalog # 100-50 (for most non-fastidious **yeast/fungi**); 50 ml x 2
If using 96 (deep) well plates and 0.5 ml per well = 48 experimental and 48 control samples

YNB medium PLUS carbon energy source:
D-glucose (U-^{13}C, 95% and 5%)
Thumb-drive containing IROA® Kit and ClusterFinder™ software instructions, access to IROA portal

Additional Materials Required: Rapidly growing cells adapted to YNB, Phosphate-buffered saline (PBS): 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2, Bradford reagent for protein determination, optional

IROA Biochemical Quantitation Kit Catalog # 200-50 (for most non-fastidious **bacteria**); 50 ml x 2
If using 96 (deep) well plates and 0.5 ml per well (5+ cell doublings plus experimental) = 48 experimental and 48 control samples

M9 Minimal medium PLUS carbon and amino acid energy sources:
D-glucose (U-^{13}C, 95% and 5%);
Amino acid mix (U-^{13}C, 95% and 5%)
DynaGuard Filters; 0.2 µm
Thumb-drive containing IROA® Kit and ClusterFinder™ software instructions, access to IROA portal

Additional Materials Required: Rapidly growing cells adapted to M9 Minimal Media, Phosphate-buffered saline (PBS): 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2, Bradford reagent for protein determination, optional

IROA Biochemical Quantitation Kit Catalog # 300-250 (for **mammalian cells**); 250 ml x 2
If 10 ml/sample (5+ cell doublings plus experimental) = 28 experimental and 28 control samples

Earle's Balanced Salt Solution/RPMI 1640 Vitamins PLUS carbon sources:
D-glucose (U-^{13}C, 95% and 5%);
Amino acid mix (U-^{13}C, 95% and 5%)
Yeast Extract (U-^{13}C, 95% and 5%)
Thumb-drive containing IROA® Kit and ClusterFinder™ software instructions, access to IROA portal

Additional Materials Required: Rapidly growing cells adapted to unlabeled Mammalian Media, dialyzed Fetal Bovine Serum (dFBS), Filtration system to sterilize final media solution, Phosphate-buffered saline (PBS): 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2, Bradford reagent for protein determination, optional

Unlabeled IROA Mammalian Media Catalog # 300-UL-250 (for testing the cell growth and division of mammalian cells); 250 ml. The mammalian medium has been found to fully support the cell growth and division of many different attached cells lines including CHO, HepG2, HC-04, HaCat, HL60, and OVAR-8.

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