IROA BIOCHEMICAL QUANTITATION KITS

IROA® Background Overview

The IROA® Biochemical Quantitation Kits are supplied with reagents and tools for simple and direct *in-vitro* labeling, identification and quantitation 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 powered components, 2) IROA ClusterFinder™ software tool, developed to characterize all peaks according to source (artifact, control, experimental), remove artifacts, identify and quantitate 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® protocol is illustrated below.

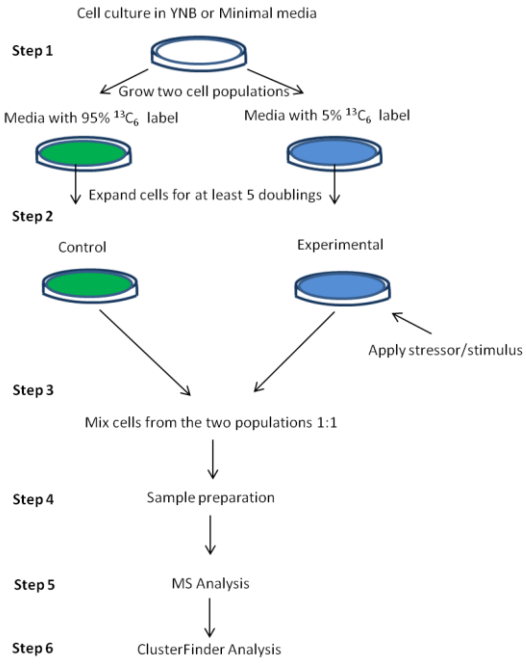
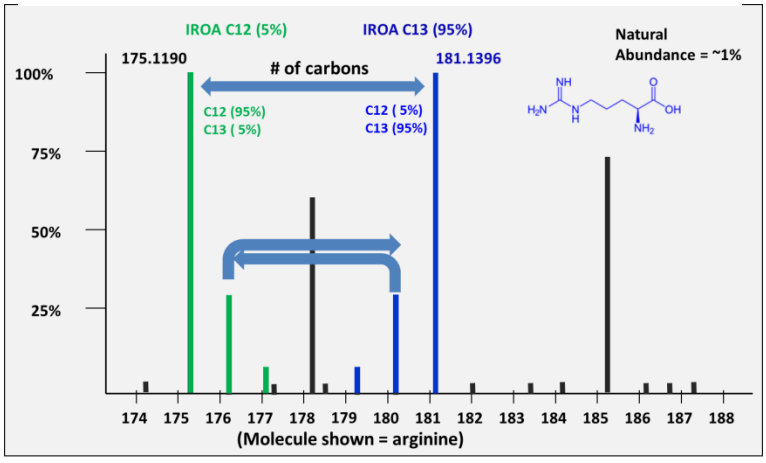
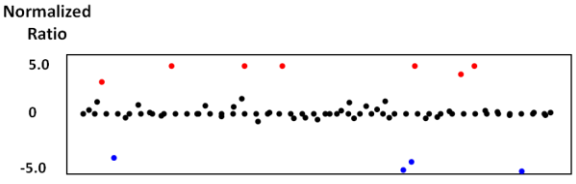


Figure: Basic IROA Protocol

The IROA envelopes. 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. Green=C12 carbon envelope; Blue=C13 carbon envelope; Black=natural abundance peaks not considered in final dataset





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. Please let us know if you would like to discuss your experimental design.

IROA Biochemical Quantitation Kit Catalog Cat. No. 100-50 (for most non-fastidious **yeast/fungi**); 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

YNB medium PLUS carbon energy source:
D-glucose (U-^{13}C, 95% and 5%)
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 Cat. No. 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%)
IROA® Kit and ClusterFinder™ software instructions, access to IROA portal

Additional Materials Required: Rapidly growing cells adapted to M9 Minimal Media (IROA Cat. No. 200-UL), Phosphate-buffered saline (PBS): 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2, Bradford reagent for protein determination, optional

Unlabeled IROA Bacterial Media Catalog Cat. No. 200-UL (for testing the cell growth and division of **bacterial** cells); 50 mL.

IROA Biochemical Quantitation Kit Catalog Cat. No. 300-250 (for **mammalian cells**); 250 mL x 2
If 10 mL/sample (5+ cell doublings plus experimental) = 28 experimental and 28 control samples
IROA 300-250 is supplied as (2) Kits: 1) **IROA PHENO-95-300 Kit** (to label the Control cell population) and, 2) the **IROA FLUX-05-300 Kit** (to label the Experimental cell population), described below

Based on 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%)
IROA® Kit and ClusterFinder™ software instructions, access to IROA portal

Additional Materials Required: Rapidly growing cells adapted to unlabeled Mammalian Media (Cat. No. IROA-300-UL), dialyzed Fetal Bovine Serum (IROA Cat No. 300-DS-75), 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

IROA PHENO-95-300 Kit (IROA® Phenotypic Quantitation Kit (U-^{13}C, 95%) for Mammalian Metabolic Profiling); 250mL
Designed for situations where it is not practical or feasible to label experimental samples such as biopsies, or large-scale fermentation. The IROA Phenotypic protocol can be applied to cell populations where specific or recommended growth media is desired or required for experimental samples, and which differs from that of the composition of the IROA media. A labeled internal Standard can be prepared by growing the same cell population (if multiple doublings can be achieved) or a cell line which closely resembles the metabolome under study in the PHENO-95-300 media. The labeled Standard can then be used (as a complex Internal Standard) to compare differences between experimental cell populations grown in the unlabeled specific growth media. ClusterFinder software is used in "Phenotypic mode" to find all labeled C13 Control Standard metabolite peaks and then locate their corresponding Experimental unlabeled C12 metabolite peak pairs. Differences in metabolite pools are quantified. ClusterFinder uses the carbon envelopes to determine the number of carbons in each metabolite and provide a molecular formula.

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

Additional Materials Required: Dialyzed fetal bovine serum (sold separately, Cat. No. 300-DS-75); Phosphate-buffered saline (PBS): 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2, Bradford reagent for protein determination, optional

Recommended: IROA unlabeled mammalian media to test cell growth (sold separately, Cat. No. IROA-300-UL)

Storage: Upon receipt, store kit at +4° C protected from light. All components are shipped at ambient temperature.

IROA FLUX-05-300 Kit (Fluxomic Quantitation Kit (U-^{13}C, 5%) for Mammalian Metabolic Profiling); 250 mL
Provides reagents to fully label mammalian cells at 5% U-^{13}C to determine the flux of a specific precursor (*e.g.* glucose, amino acids, etc.) enriched in 95% or 99% U-^{13}C that are introduced into the metabolic system under study. The use of 5% provides a mechanism to identify all metabolic pools and exclude all non-biological signals and is completely unbiased. The flow of the tracer into any metabolic pool will be determined by the presence of a perturbation of the shape of the 5% ^{13}C envelope. All of the molecular identification aspects of IROA are still supported by the ClusterFinder software (Fluxomic Mode"). The automation of data collection and the ability to measure flux as a function of a time-course are easily accomplished using this protocol.

Based on Earle's Balanced Salt Solution/RPMI 1640 Vitamins PLUS carbon sources:
D-glucose (U-^{13}C, 5%)

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info@iroatech.com

Amino acid mix (U-^{13}C, 5%)
Yeast Extract (U-^{13}C, 5%)
IROA® Kit and ClusterFinder™ software instructions, access to IROA portal

Required: Dialyzed fetal bovine serum (sold separately, Cat. No. 300-DS-75)

Additional Materials Recommended: IROA unlabeled mammalian media to test cell growth (sold separately, Cat. No. IROA-300-UL), Phosphate-buffered saline (PBS): 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2, Bradford reagent for protein determination, optional

Storage: Upon receipt, store kit at +4° C protected from light. All components are shipped at ambient temperature.

Unlabeled IROA Mammalian Media Catalog Cat. No. 300-UL (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 (see IROAtech.com for complete list).