AbsoluteIDQ® p180 Kit
Targeted Metabolite Identification and Quantification

Bringing our targeted metabolomics expertise to your lab.

The Biocrates AbsoluteIDQ p180 mass spectrometry Assay Preparation Kit delivers the widest range of metabolomic information available from a single targeted assay, covering a large number of key metabolites from main metabolic pathways. The metabolome – the complete set of small molecule metabolites arising from biochemical processes in various compartments of organisms – is dynamic and complex. The AbsoluteIDQ p180 Kit quantitatively analyzes a large number of metabolites that have already been identified as part of key biochemical pathways, providing fundamental data to link changes in the metabolome to biological events.

Highlights

• Quantifies up to 188 metabolites in five compound classes
  – Acylcarnitines
  – Amino Acids
  – Biogenic Amines
  – Hexoses (sum of Hexoses)
  – Phospho- and Sphingolipids
    Phosphatidylcholines
    Lyso-Phosphatidylcholines
    Sphingomyelins

• Isotope-labeled and chemically homologous internal standards are used for quantification
  – 53 analytes are fully validated as absolutely quantitative
  – Majority of internal standards is pre-pipetted on the Kit plate
  – Calibration standard mix in 7 different concentrations is included

• Controls included (3 different concentration levels)

• Patented plate design ensures efficient sample derivatization and excellent reproducibility in analyte extraction

• Standardized assay in 96-well plate format
  – Suitable for manual and automated, high throughput operation
  – Very small sample volume of 10 µL
  – Allows in-house handling of valuable samples and proprietary data

Key Features

• Combined flow injection/liquid chromatography assay

• Designed to be used with Triple quadrupole mass spectrometers
  – Industry leading triple quadrupole and hybrid quadrupole ion trap platforms provide broad linear dynamic range
  – Enables accurate and precise quantification analyses
  – Facilitates monitoring of 100’s of MRM pairs, for wide compound coverage in a single injection

• MS analysis by FIA-MS/MS method, for acylcarnitines, hexoses, phospho- and shingolipids
  – Ionization via Electrospray Ionization (ESI) source
  – Multiple Reaction Monitoring (MRM) mode for high specificity and sensitivity
  – Rapid and robust assay, simple work flow
  – Established analysis method (following newborn screening for inborn defects)
  – Employs carefully selected MRM pairs for quantification

• MS analysis by LC-MS/MS method for amino acids and biogenic amines
  – Robust reversed-phase column
  – Employs carefully selected MRM pairs for quantification

• 82 samples per plate

• Includes MetIDQ™ Software for managing the entire Kit workflow and for automated calculation of metabolite concentrations
  – Optional MetIDQ™ StatPack Software Module for full statistical analysis available
  – Optional MetIDQ™ RatioExplorer Software Module for comprehensive evaluation of results from metabolomic studies supported by the calculation of sums and ratios of selected metabolites or metabolic classes.

• On-site application support for instrumentation setup, Kit preparation and software training available
Assay Workflow

The workflow of the Absolute/DQ assay is controlled by the MetaDQ™ Software, which is an integral part of the Kit. Four integrated modules, MetLIMS, MetConc, MetVal and MetStat, handle individual steps in the process, from project setup and sample registration to data processing. The scheme on the right gives an overview of the Kit workflow.

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Register the Assay in MetLIMS</td>
<td>30 min.</td>
<td>Import and register samples, generate 96-well plate overview and MS batch file for MS-specific software</td>
</tr>
<tr>
<td>2. Assay preparation</td>
<td>3 - 4 h</td>
<td>Prepare reagents and perform assay in the laboratory</td>
</tr>
<tr>
<td>3. Process Assay in the Mass Spectrometer</td>
<td>30 h (HPLC + FIA) 16 h (UHPLC + FIA)</td>
<td>Instrument-specific acquisition methods are provided 3a. Perform LC-MS run (analysis of amino acids and biogenic amines) 3b. Perform FIA-MS run (analysis of lipids, acylcarnitines and hexose)</td>
</tr>
<tr>
<td>4. LC-MS-based quantitation in Analyst software</td>
<td>3 - 4 h</td>
<td>Calculation of analyte concentrations, manual check of peak integration (Concentrations of calibration standards and quantitation methods are provided)</td>
</tr>
<tr>
<td>5. Convert Mass Spectrometer Data</td>
<td>15 min.</td>
<td>MS data files (FIA-MS) are imported and the concentrations are automatically calculated. LC-MS-based concentrations are imported from the instrument-specific results file.</td>
</tr>
<tr>
<td>6. Validate the Kit Plate</td>
<td>20 min.</td>
<td>Automated quality assessment of Biocrates calibration standards, quality controls and internal standards.</td>
</tr>
<tr>
<td>7. Evaluate and Export Data</td>
<td>20 min.</td>
<td>The results (concentration values for metabolites) are evaluated and can be exported in various file formats</td>
</tr>
<tr>
<td>Optional Module</td>
<td></td>
<td>8. Statistical Analysis (optional)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Box plots, Scatter plots, univariate tests (p-values), ROC curves, PCA</td>
</tr>
</tbody>
</table>

Registering the Assay

The MetaLIMS component is a flexible Laboratory Information Management System for the

- Registration of samples and projects
- Administration of blanks, standards and quality controls
- Import of external sample lists

The software automatically generates a MS-specific file containing all sample information and the injection sequence, which is loaded into the mass spectrometer software to generate the acquisition batch. Additional MetaLIMS features include:

- Generation of work lists for manual or automatic sample preparation
- Addition of detailed information to samples, to streamline subsequent statistical analysis
- Barcode printing
Assay Preparation

The unique design of the AbsoluteIDQ Kit plate ensures quick, easy and reproducible sample preparation and efficient metabolite extraction from biological samples. By incorporating the internal standards into each well of the plate, many potential sources of error in quantifying the metabolites have already been eliminated. The patented plate is designed to perform all sample preparation steps directly on the 96-well plate.

The total assay preparation time of 3-4 h includes the time required for pipetting, derivatization and drying of the samples. The actual hands-on time required for preparation of a 96-well plate is only about 90 minutes.

Processing the Assay in the Mass Spectrometer

Sample injections and LC-MS analysis can be performed with all autosamplers and binary HPLC systems. A dummy plate for easy setup of the autosampler is included. MS-specific acquisition methods are provided. In the FIA-MS/MS method, each extracted sample is analyzed by a 3 min run in both positive and negative ion mode:

- 158 MRM pairs measured in positive ion mode (20 IS)
- 2 MRM pairs measured in negative ion mode (1 IS)
- 2 x 20 µL injection, flow rate of 30 µL/min
- Total running time per sample about 7 min

In the LC-MS/MS method, each extracted sample is chromatographically separated and analyzed in positive ion mode.

- 71 MRM pairs measured in positive ion mode (25 IS)
- 1 x 10 µL injection, flow rate of 500 µL/min (HPLC) or 900 µL/min (UHPLC)

LC-MS/MS-based Quantification with MS-Instrument Specific Software

The calculation of metabolite concentrations after the LC-MS/MS run is performed in the mass spectrometer software:

- Concentrations of calibration standards and quantification methods are provided
- Only a manual check of peak integration is recommended
Converting Mass Spectrometer Data

After the FIA-MS/MS run, nearly 15,000 concentration values are automatically calculated by the MetIDQ™ Software for one Kit plate. The MetConc module interacts with MS-specific software and:

- Reads and imports data files into the MetIDQ database
- Automated algorithms simultaneously calculate final metabolite concentrations
- Total Ion Count (TIC) is used to define the time range for quantification for all MRM pairs

The results from the LC-MS/MS run (obtained by MS software) are imported into the MetIDQ database for further analysis together with the data from the FIA run.

Validating the Kit Plate

An automatic quality assessment of the data is performed by the MetVal module. It checks the obtained values for:

- Blanks
- Internal standards
- Quality controls

with the ranges set in the operating procedure of the method. Values outside the defined ranges are highlighted, and this information is also transferred to the results table.

The results can be visualized in numerous graphical layouts, giving a quick but comprehensive overview of accuracy and quality of the results.

Evaluating and Exporting the Data

The MetStat module summarizes the assay results. It offers a variety of display options and a tool for data normalization.

In a final step, the concentration data can be exported in various file formats (csv, xls, xlsx, xml, txt) for further statistical or bioinformatic analysis in other programs (e.g., R).
Method Validation

The AbsoluteIDQ Kit method has been validated according to the FDA Guidance for Industry - Bioanalytical Method Validation. Tests of accuracy and precision have been performed. Coefficients of variation (CVs) have been determined in experiments performed in various external laboratories and on different days. The CVs are generally well below 15% (upper panel) and show good consistency across different labs and different days, yielding robust and reproducible values. Further tests using defined commercially available reference plasma controls showed excellent accuracy as illustrated for amino acids determined with the AbsoluteIDQ p180 Kit (lower panel).

Main Application Areas

The AbsoluteIDQ p180 Kit covers an even wider range of metabolite classes than the AbsoluteIDQ p150 Kit. In addition, it includes a set of biogenic amines, which play a central role in cellular growth and/or proliferation processes. Considering cancer as an example, rapidly proliferating tissues usually show high concentrations of polyamines. With this broad metabolite spectrum, the application areas of the AbsoluteIDQ p180 Kit are diverse. Besides basic research, major areas of application are pharmaceutical and clinical research. Metabolomic methods are also well suited for translational research studies. Unlike a transcript or protein, a given metabolite is the same in every organism, with the central metabolic pathways generally conserved during evolution. Therefore, studies from cell culture to preclinical animal models and clinical trials can be performed using the same analytical method. In addition, metabolomics has varied applications in the food industry and nutrition research, investigating health effects of functional food as well as effect of environment and exercise.

<table>
<thead>
<tr>
<th>Metabolite Class</th>
<th>Biological Relevance (selected examples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acylcarnitines</td>
<td>Energy metabolism, fatty acid transport and mitochondrial fatty acid oxidation (e.g., inborn disorders such as MCAD), ketosis, oxidative stress, mitochondrial membrane damage (apoptosis)</td>
</tr>
<tr>
<td>Amino Acids</td>
<td>Amino acid metabolism (e.g., inborn disorders such as PKU, MSUD), urea cycle, activity of gluconeogenesis and glycolysis, insulin sensitivity/resistance, neurotransmitter metabolism, oxidative stress</td>
</tr>
<tr>
<td>Biogenic Amines</td>
<td>Neurological disorders, cell proliferation, cell cycle progression, DNA stability, oxidative stress</td>
</tr>
<tr>
<td>Hexoses</td>
<td>Carbohydrate metabolism</td>
</tr>
<tr>
<td>Phosphatidylcholines</td>
<td>Dyslipidemia, membrane composition and damage, fatty acid profile, activity of desaturases</td>
</tr>
<tr>
<td>Lyso-Phosphatidylcholines</td>
<td>Degradation of phospholipids (phospholipase activity), membrane damage, signaling cascades, fatty acid profile</td>
</tr>
<tr>
<td>Sphingomyelins</td>
<td>Signaling cascades, membrane damage (e.g., neurodegeneration)</td>
</tr>
</tbody>
</table>
Conclusion

The **AbsoluteIDQ p180** Kit is an easy to use and accurate method for quantifying a high number of already identified endogenous intermediates or final metabolites of key biochemical pathways. The assay requires very small sample amounts (10 µL) and shows excellent reproducibility. The integrated MetIDQ Software streamlines data analysis by automated calculation of metabolite concentrations.

Based on targeted metabolomics, the **AbsoluteIDQ p180** Kit not only eliminates the need for metabolite identification, it also provides quantitative and annotated data. Whether discovering biomarkers or monitoring metabolic pathways, this fast and reliable assay is an excellent choice for researchers who are looking for a cost-effective solution to measure metabolites under quality-controlled conditions in their own laboratory, even at a high throughput scale.

Kit Contents

<table>
<thead>
<tr>
<th>Kit Item</th>
<th>Description</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>AbsoluteIDQ Kit Plate</td>
<td>A 96 deepwell plate plus a filter plate attached with sealing tape</td>
<td>Sealed under nitrogen in a plastic bag</td>
</tr>
<tr>
<td>Silicone mat cover for plate</td>
<td>Covers the plate after sample preparation, 2 items</td>
<td></td>
</tr>
<tr>
<td>Biocrates Solvent I</td>
<td>Sealed glass ampoule, 2 items</td>
<td>Used as FIA running solvent</td>
</tr>
<tr>
<td>Biocrates Quality Controls</td>
<td>3 vials</td>
<td></td>
</tr>
<tr>
<td>Biocrates Standards</td>
<td>7 vials</td>
<td>Used as calibration standards for the LC-MS/MS assay</td>
</tr>
<tr>
<td>Biocrates Internal Standards</td>
<td>1 vial</td>
<td></td>
</tr>
<tr>
<td>96 deepwell capture plate</td>
<td>For diluting sample extracts obtained after Kit preparation</td>
<td></td>
</tr>
</tbody>
</table>

Ordering Information

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>AbsoluteIDQ p180 Kit - (96)</td>
<td>96 Tests</td>
</tr>
<tr>
<td>AbsoluteIDQ p180 Kit - (56)</td>
<td>56 Tests</td>
</tr>
<tr>
<td>MetIDQ StatPack Module</td>
<td>each</td>
</tr>
<tr>
<td>MetIDQ Ratio Explorer Module</td>
<td>each</td>
</tr>
</tbody>
</table>