Metabolic Phenotyping Kit for Mass Spectrometry Based Quantitation of up to 188 Metabolites
Bringing Our Targeted Metabolomics Expertise to Your Lab

The mass spectrometry based AbsoluteIDQ® p180 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 (40)
  - Amino acids and Biogenic amines (41-42)
  - Hexoses (sum of Hexoses) (1)
  - Glycerophospholipids (88-90) and Sphingolipids (14-15)
    - 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

Assay Workflow

1. Register the Assay in MetLIMS
   Import and register samples, generate 96-well plate overview and MS batch file for MS-specific software

2. Assay Preparation
   Prepare reagents and perform assay in the laboratory

   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, acylcarnitins and hexose)

4. LC-MS-Based Quantitation
   Calculation of analyte concentrations, manual check of peak integration (Concentrations of calibration standards and quantitation methods are provided)

5. Convert Mass Spectrometer Data
   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.

6. Validate the Kit Plate
   Automated quality assessment of Biocrates calibration standards, quality controls and internal standards.

7. Evaluate and Export Data
   The results (concentration values for metabolites) are evaluated and can be exported in various file formats

Optional Module

8. Statistical Analysis (optional)
   Box plots, Scatter plots, univariate tests (p-values), ROC curves, PCA

Total: 25h (UHPLC) - 35h (HPLC)
Main Application Areas

The AbsoluteIDQ® p180 Kit covers a range of metabolite classes including 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 (e.g. cancer research, neuroscience research). Metabolomics-based 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>Metabolites</th>
<th>Metabolite Class(es)</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine, Glycine, Leucine, Valine, Carnosine, L-DOPA</td>
<td>Amino acids, Biogenic amines, Phosphatidylcholines</td>
<td>Neurodegenerative diseases (e.g. Alzheimer’s disease; Parkinson’s disease; Huntington’s disease)</td>
</tr>
<tr>
<td>Alanine, Glutamine, Glutamic acid, Glycine, Creatine, Proline taurine, Valine, Asparagine, Lysine, and many others</td>
<td>Amino acids, Biogenic amines, Lysophosphatidylcholines</td>
<td>Cancer</td>
</tr>
<tr>
<td>Isoleucine, Leucine, Phenylalanine, Tyrosine, Valine, Creatinine, PC aa C34:4, SM C14:0, Glucose</td>
<td>Amino acids, Biogenic amines, Phosphatidylcholines, Sphingomyelins, Hexoses</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Alanine, Aspartate, Citrate, Creatinine, Fructose, Glucose, Glutamine, Glycine, Histidine, Isoleucine, Proline, Serine, Threonine, Tryptophan, Valine</td>
<td>Amino acids, Hexoses</td>
<td>Cardiovascular disease</td>
</tr>
</tbody>
</table>


Kit Parameters

**Total Runtime (for 96 tests):**
- HPLC: up to 36 h (4 h sample prep & assay registration in MetIDQ™ + up to 28 h LC- and FIA-MS/MS + 3 h data processing + 1 h data validation and result export in MetIDQ™)
- UHPLC: up to 29 h (4 h sample prep & assay registration in MetIDQ™ + up to 21 h LC- and FIA-MS/MS + 3 h data processing + 1 h data validation and result export in MetIDQ™)

**Sample matrix:**
- Human plasma*, human serum, animal plasma or serum, tissue extracts of different species, cell lysate, cell culture supernatant, CSF, urine (with restrictions), feces (human tested), BALF, and further fluid biological matrices (please ask customer support)
- Citrate plasma is not accepted

**LC-MS/MS platforms:**
- AB SCIEX: 4000 series*, 4500 series, API 5000™ and 5500 series
- Waters: TQ MS™ and TQ S™
- Thermo: TQ Vantage™

**Ionization:**
- ESI positive and negative

**Sample preparation:**
- On Biocrates 96-well filter plate, 3-4 h
- Up to 82 samples per Kit

**Sample volume:**
- 10 μL (most matrices like plasma or serum)

**MS runtime:**
- HPLC: up to 17.5 min / sample (LC + FIA)
- UHPLC: up to 13 min / sample (LC + FIA)

**Quantitation:**
- LC: 7 external calibration standards
- Isotope labeled internal standards for most analytes
- FIA: Isotope labeled internal standards for representative analytes

**Quality controls:**
- 3 levels: low, medium, high
- Human plasma based

* used for Kit validation
AbsoluteIDQ® p180 Kit – Your 1st Choice for Biomarker Discovery

The p180 Kit offers the possibility to identify potential biomarkers for diagnostic investigation as well as drug therapy monitoring by providing tools for a comprehensive insight into pathways and metabolic signatures of diseases (such as cancer or neurodegenerative diseases).

The Kit requires very small sample amounts (10 μL) and shows excellent reproducibility and can be used with a wide range of species and biological matrices.

An integrated software solution (MetIDQ™) is used to manage the entire analysis workflow, while enhancing the standardization and harmonization of project data processing.

Biocrates’ Kits can be used in your own laboratory or as analytical services at Biocrates’ corporate headquarters.

Kit Content

<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>
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<tr>
<td>Biocrates® Internal Standards</td>
<td>1 vial</td>
<td></td>
</tr>
<tr>
<td>96 deepwell capture plate</td>
<td></td>
<td>For diluting sample extracts obtained after Kit preparation</td>
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<tr>
<td>USB memory stick</td>
<td>- MetIDQ™ Software</td>
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<td></td>
<td>- OracleXE (Express Edition) database</td>
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<tr>
<td></td>
<td>- Acquisition and quantification methods</td>
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<tr>
<td></td>
<td>- User Manual and Analytical Specifications</td>
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<tr>
<td></td>
<td>- StatPack User Manual</td>
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BIOCRATES Life Sciences AG
Ordering Information

<table>
<thead>
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<tbody>
<tr>
<td>AbsoluteIDQ® p180 Kit - (96)</td>
<td>Contact our sales department</td>
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<tr>
<td>AbsoluteIDQ® p180 Kit - (56)</td>
<td>Contact our sales department</td>
</tr>
<tr>
<td>MetIDQ™ StatPack Module</td>
<td>9120052120189</td>
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<tr>
<td>MetIDQ™ Ratio Explorer Module</td>
<td>9120052120899</td>
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