

Absolute/DQ® p180 Kit

Supporting Biomarker Discovery in Preclinical and Clinical Research



Metabolic Phenotyping Kit for Mass Spectrometry Based

Quantitation of up to 188 Metabolites

n(CH±):

Bringing Our Targeted Metabolomics Expertise to Your Lab

The mass spectrometry based Absolute/*IDQ*® 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 compart-

ments of organisms – is dynamic and complex. The Absolute *IDQ*® 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

30 min.

3 - 4 h

up to 28 h (HPLC + FIA) up to 21 h (UHPLC + FIA)

3 - 4 h

15 min.

20 min. 20 min. 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

3. Process Assay in the Mass Spectrometer 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 Absolute *IDQ*® 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 Absolute *IDQ*® 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.

Metabolites	Metabolite Class(es)	Disease
Alanine, Glycine, Leucine, Valine, Carnosine, L-DOPA	Amino acids, Biogenic amines, Phosphatidylcholines	Neurodegenerative diseases (e.g. Alzheimer's disease; Parkinson's disease; Huntington's disease)
Alanine, Glutamine, Glutamic acid, Glycine, Creatine, Proline taurine, Valine, Asparagine, Lysine, and many others	Amino acids, Biogenic amines, Lysophosphatidylcholines	Cancer
Isoleucine, Leucine, Phenylalanine, Tyrosine, Valine, Creatinine, PC aa C34:4, SM C14:0, Glucose	Amino acids, Biogenic amines, Phosphatidylcholines, Sphingo- myelins, Hexoses	Diabetes
Alanine, Aspartate, Citrate, Creatinine, Fructose, Glucose, Glutamine, Glycine, Histidine, Isoleucine, Proline, Serine, Threonine, Tryptophan, Valine	Amino acids, Hexoses	Cardiovascular disease

^{*}Metabolomics and the Diagnosis of Human Diseases - A Guide to the Markers and Pathophysiological Pathways Affected, S. Medina et al., 2014.

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: TSQ 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





Absolute/DQ® 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 μ L) and shows excellent reproducibility and can be used with a wide range of species and biological matrices.

An integrated software solution ($Met \textit{IDQ}^{TM}$) 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 our as analytical services at Biocrates' corporate headquarters.

Kit Content

Kit Item	Description	Details
Absolute <i>IDQ</i> ® Kit Plate	A 96 deepwell plate plus a filter plate attached with sealing tape	Sealed under nitrogen in a plastic bag
Silicone mat cover for plate	Covers the plate after sample preparation, 2 items	
Biocrates® Solvent I	Sealed glass ampoule, 2 items	Used as FIA running solvent
Biocrates® Quality Controls	3 vials	
Biocrates® Standards	7 vials	Used as calibration standards for the LC-MS/MS assay
Biocrates® Internal Standards	1 vial	
96 deepwell capture plate		For diluting sample extracts obtained after Kit preparation
USB memory stick	-MetIDQ™ Software -OracleXE (Express Edition) database -Acquisition and quantification methods -User Manual and Analytical Specifications -StatPack User Manual -RatioExplorer User Manual	

BIOCRATES Life Sciences AG Ordering Information

Product Description	
Absolute <i>IDQ®</i> p180 Kit - (96)	Contact our sales department
Absolute <i>IDQ</i> ® p180 Kit - (56)	Contact our sales department
Met <i>IDQ</i> ™ StatPack Module	9120052120189
Met <i>IDQ</i> ™ Ratio Explorer Module	9120052120899

For Further Information, Please Contact:

BIOCRATES Life Sciences AG Eduard-Bodem-Gasse 8 6020 Innsbruck, Austria

phone: +43.512.579 823 fax: +43.512.579 823 329 email: sales@biocrates.com www.biocrates.com