

Absolute/IDQ™ Kit: Targeted Metabolomics Identification and Quantification

Accurate, Reproducible, Metabolomics Studies

The word metabolome refers to the complete set of small molecule metabolites arising from specific cellular processes that are on-going in biological cells, tissues, organs or organisms. Studying the metabolome can lead to an instantaneous snapshot into the physiology of the cell or organism. While the metabolome is dynamic and complex, there are a large number of metabolites that are already identified and understood as intermediates or end products of key biological pathways. This fact makes a targeted approach to metabolomics an excellent strategy for many areas of research. Targeted assays typically enable higher throughput, higher quality quantitation and easier down-stream data processing. Additionally, biological answers can often be obtained in a more rapid, seamless manner

AB SCIEX Triple Quad™ and QTRAP® systems are ideal instruments for targeted metabolomics workflows using Multiple Reaction Monitoring (MRM). With the unique LINAC® Collision Cell, large numbers of analytes can be analyzed across a broad dynamic range with high accuracy and precision.



Features of Absolute/IDQ™ Kits for Targeted Metabolomics

- Accurately identifies and quantifies over 160 metabolites in only a few minutes per sample (Table 2)
- Covers four classes of metabolites (Table 1)
 - 1. Amino acids
 - 2. Acylcarnitines
 - 3. Glycerophospho- and Sphingolipids
 - 4. Hexose
- Requires very small sample volumes (10 µL)
- Includes software to control sample management, data collection, data validation, and analysis

Absolute/IDQ™ Kit Contents

- Absolute/IDQ™ kit plate
- Silicone mat cover for kit plate
- Reagent buffer vials
- BIOCRATES solvent I
- 96 deep-well plate with attached filter plate
- Standards and quality control (QC) samples
- Kit user manual
- Analytical specifications
- Met/IQ™ Software
- AB SCIEX MS acquisition methods

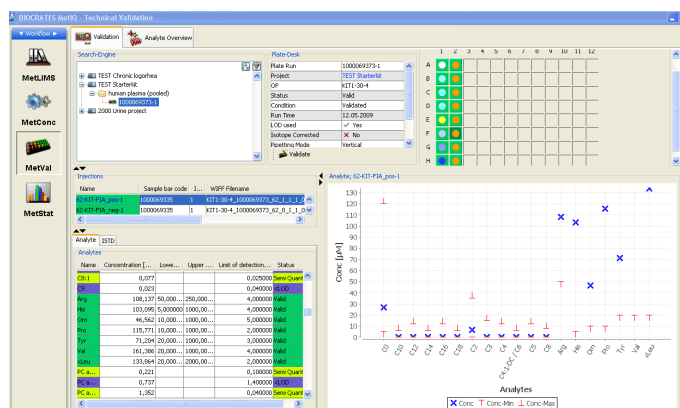


Figure 1. MetVal™ Module in the Met/IQ™ Software. Evaluation of analyte concentrations in human plasma is shown.

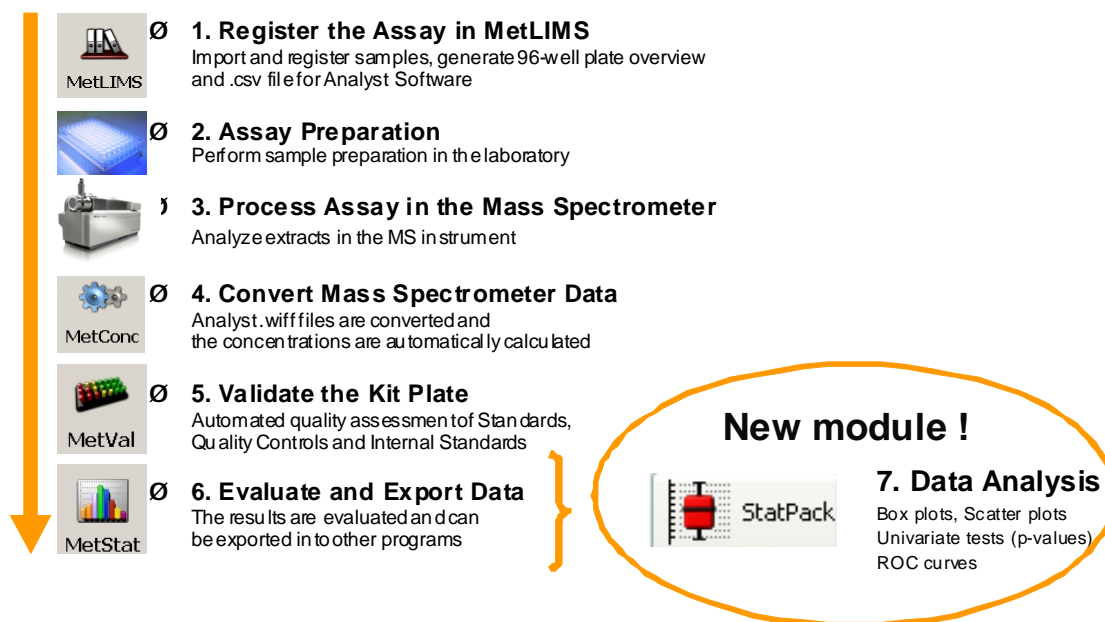


Figure 2. Absolute/DQ™ Kit Workflow. The kit provides the whole workflow, from sample preparation, through data acquisition to data processing and reporting.

The complete workflow for the Absolute/DQ™ kit is shown in Figure 2. The assay preparation is very simple. Samples are added to the upper filter spot on the specially adapted 96-well kit plate. Internal standards are incorporated into each well of this plate. Phenylisothiocyanate (PITC) is then added to each well to derivatize any amino acids. The plate is then dried and the metabolites are extracted with the organic extraction solvent. After centrifugation, the lower deep well capture plate containing the metabolites is analyzed by LC/MS/MS. Only about 60 minutes of hands-on preparation time is required for each 96-well microtitre plate. Each sample is then analyzed by a 3 minute flow injection analysis (FIA) in both positive and negative ion mode enabling a fast run time for the entire plate.

Combined with the AB SCIEX Triple Quad or QTRAP® system, the Absolute/DQ kits enables high throughput, quantitative, targeted metabolomics experiments to be performed routinely in any laboratory setting.

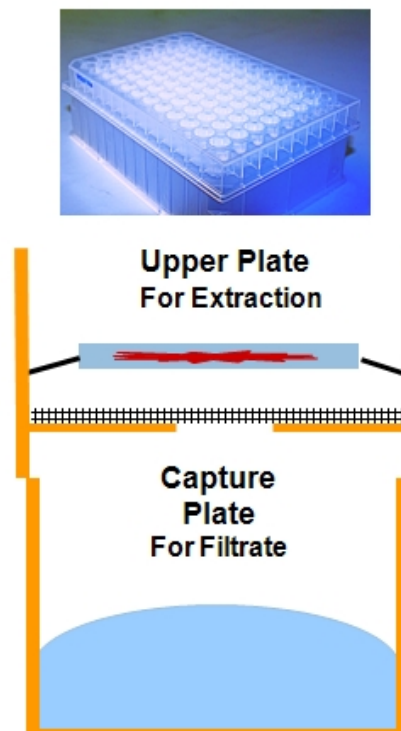


Figure 3. Unique Design of the Absolute/DQ™ Kit Plate. These plates are designed so that all sample preparation is performed directly within the microtitre plate. This simplifies and streamlines the workflow, making the assay amenable to either manual or automated sample preparation.

Table 1. Overview of Metabolite Classes. A number of major classes of metabolites are covered in the Absolute/IDQ™ Kit. Details of analyzed metabolites in each class are listed in Table 2.

Metabolite Class	Number of Analytes	
	Measured	Biological Relevance (selected examples)
Acylcarnitine	41	Energy metabolism, fatty acid transport and mitochondrial fatty acid oxidation (eg. Inborn disorders like MCAD), ketosis, oxidative stress, mitochondrial membrane damage (apoptosis)
Amino Acids	13 Proteinogenic Amino Acids + Ornithine	Amino acid metabolism (eg. Inborn disorders like PKU, MSUD), urea-cycle, activity of gluconeogenesis and glycolysis, insulin sensitivity / resistance, neurotransmitter metabolism, oxidative stress
Hexose	Sum of Hexose (90-95% Glucose)	Carbohydrate metabolism
Lyso - Phosphatidylcholine	15	Degradation of phospholipids (phospholipase activity), membrane damage, signaling cascades, fatty acid profile
Phosphatidylcholine	77	Dyslipidemia, membrane composition and damage, fatty acid profile, activity of destaurases
Sphingomyelin	15	Signalling cascades, membrane damage (eg. Neurodegeneration)

Met/Q™ Software

Included with each kit, BIOCRATES software converts complex metabolomics data into powerful clinical knowledge. The Met/Q™ Software package is an integral part of the Absolute/IDQ™ solution and provides an integrated solution for the complete targeted metabolomics analytical process. Four separate modules, MetLIMS, MetConc, MetVal and MetStat handle separate steps in the process, from project setup to data conversion, to calculation of metabolite concentrations.

- MetLIMS – laboratory management system to define projects, import sample information, administer standards and quality controls, print barcodes.
- MetConc – automatically reads Analyst® Software data files and calculates metabolite concentrations.
- MetVal – validates datasets by analyzing internal standards, blanks, quality controls, and full plate results.
- MetStat – finalizes results for the project by combining unknowns and validation results. Multiple data display and export options are available.

Conclusions

BIOCRATES and AB SCIEX have teamed up to deliver a metabolomic assay preparation kit with a simplified workflow that produces a robust assay and reliable data. The Absolute/IDQ™ Kit, sold and supported exclusively by BIOCRATES Life Sciences AG, is designed for use on the AB SCIEX API 4000™, 4000 QTRAP®, API 5000™, AB SCIEX Triple Quad™ 5500, and QTRAP® 5500 systems. The Absolute/IDQ Kit, combined with AB SCIEX instrumentation delivers the deepest and broadest metabolomic information available from a single targeted assay, enabling you to accelerate your research while simultaneously reducing your cost of performing targeted, quantitative metabolomics.

Table 2. List of Analyzed Metabolites.

Aminoacids			Glycerophospholipids			
42	Arg-PTC	Arginine	57	lysoPC a C6:0	103	PC aa C40:6
43	Gln-PTC	Glutamine	58	lysoPC a C14:0	104	PC aa C42:0
44	Gly-PTC	Glycine	59	lysoPC a C16:0	105	PC aa C42:1
45	His-PTC	Histidine	60	lysoPC a C16:1	106	PC aa C42:2
46	Met-PTC	Methionine	61	lysoPC a C17:0	107	PC aa C42:4
47	Orn-PTC	Ornithine	62	lysoPC a C18:0	108	PC aa C42:5
48	Phe-PTC	Phenylalanine	63	lysoPC a C18:1	109	PC aa C42:6
49	Pro-PTC	Proline	64	lysoPC a C18:2	110	PC ae C30:0
50	Ser-PTC	Serine	65	lysoPC a C20:3	111	PC ae C30:1
51	Thr-PTC	Threonine	66	lysoPC a C20:4	112	PC ae C30:2
52	Trp-PTC	Tryptophan	67	lysoPC a C24:0	113	PC ae C32:1
53	Tyr-PTC	Tyrosine	68	lysoPC a C26:0	114	PC ae C32:2
54	Val-PTC	Valine	69	lysoPC a C26:1	115	PC ae C34:0
55	xLeu-PTC	xLeucine	70	lysoPC a C28:0	116	PC ae C34:1
Hexoses			71	lysoPC a C28:1	117	PC ae C34:2
56	H1	Hexose	72	PC aa C24:0	118	PC ae C34:3
			73	PC aa C26:0	119	PC ae C36:0
Sphingolipids			74	PC aa C28:1	120	PC ae C36:1
149	SM (OH) C14:1		75	PC aa C30:0	121	PC ae C36:2
150	SM C16:0		76	PC aa C30:2	122	PC ae C36:3
151	SM C16:1		77	PC aa C32:0	123	PC ae C36:4
152	SM (OH) C16:1		78	PC aa C32:1	124	PC ae C36:5
153	SM C18:0		79	PC aa C32:2	125	PC ae C38:0
154	SM C18:1		80	PC aa C32:3	126	PC ae C38:1
155	SM C20:2		81	PC aa C34:1	127	PC ae C38:2
156	SM C22:3		82	PC aa C34:2	128	PC ae C38:3
157	SM (OH) C22:1		83	PC aa C34:3	129	PC ae C38:4
158	SM (OH) C22:2		84	PC aa C34:4	130	PC ae C38:5
159	SM C24:0		85	PC aa C36:0	131	PC ae C38:6
160	SM C24:1		86	PC aa C36:1	132	PC ae C40:0
161	SM (OH) C24:1		87	PC aa C36:2	133	PC ae C40:1
162	SM C26:0		88	PC aa C36:3	134	PC ae C40:2
163	SM C26:1		89	PC aa C36:4	135	PC ae C40:3
			90	PC aa C36:5	136	PC ae C40:4
			91	PC aa C36:6	137	PC ae C40:5
			92	PC aa C38:0	138	PC ae C40:6
			93	PC aa C38:1	139	PC ae C42:0
			94	PC aa C38:3	140	PC ae C42:1
			95	PC aa C38:4	141	PC ae C42:2
			96	PC aa C38:5	142	PC ae C42:3
			97	PC aa C38:6	143	PC ae C42:4
			98	PC aa C40:1	144	PC ae C42:5
			99	PC aa C40:2	145	PC ae C44:3
			100	PC aa C40:3	146	PC ae C44:4
			101	PC aa C40:4	147	PC ae C44:5
			102	PC aa C40:5	148	PC ae C44:6

Table 2. List of Analyzed Metabolites, continued.

Acylcarnitines					
1	C0	DL-Carnitine	21	C10	Decanoyl-L-carnitine
2	C2	Acetyl-L-carnitine	22	C10:1	Decenoyl-L-carnitine
3	C3	Propionyl-L-carnitine	23	C10:2	Decadienyl-L-carnitine
4	C3:1	Propenoyl-L-carnitine	24	C12	Dodecanoyl-L-carnitine
5	C3-OH	Hydroxypropionyl-L-carnitine	25	C12:1	Dodecenoyl-L-carnitine
6	C4	Butyryl-L-carnitine	26	C12-DC	Dodecanedioyl-L-carnitine
7	C4:1	Butenyl-L-carnitine	27	C14	Tetradecanoyl-L-carnitine
8	C4-OH (C3-DC)	Hydroxybutyryl-L-carnitine (Malonyl-L-carnitine)	28	C14:1	Tetradecenoyl-L-carnitine
9	C5	Valeryl-L-carnitine	29	C14:1-OH	Hydroxytetradecenoyl-L-carnitine
10	C5:1	Tiglyl-L-carnitine	30	C14:2	Tetradecadienyl-L-carnitine
11	C5:1-DC	Glutaconyl-L-carnitine	31	C14:2-OH	Hydroxytetradecadienyl-L-carnitine
12	C5-DC (C6-OH)	Glutaryl-L-carnitine (Hydroxyhexanoyl-L-carnitine)	32	C16	Hexadecanoyl-L-carnitine
13	C5-M-DC	Methylglutaryl-L-carnitine	33	C16:1	Hexadecenoyl-L-carnitine
14	C5-OH (C3-DC-M)	Hydroxyvaleryl-L-carnitine (Methylmalonyl-L-carnitine)	34	C16:1-OH	Hydroxyhexadecenoyl-L-carnitine
15	C6 (C4:1-DC)	Hexanoyl-L-carnitine (Fumaryl-L-carnitine)	35	C16:2	Hexadecadienyl-L-carnitine
16	C6:1	Hexenoyl-L-carnitine	36	C16:2-OH	Hydroxyhexadecadienyl-L-carnitine
17	C7-DC	Pimelyl-L-carnitine	37	C16-OH	Hydroxyhexadecanoyl-L-carnitine
18	C8	Octanoyl-L-carnitine	38	C18	Octadecanoyl-L-carnitine
19	C8:1	Octenoyl-L-carnitine	39	C18:1	Octadecenoyl-L-carnitine
20	C9	Nonanoyl-L-carnitine	40	C18:1-OH	Hydroxyoctadecenoyl-L-carnitine
			41	C18:2	Octadecadienyl-L-carnitine

Availability

The Absolute/IDQ™ Metabolomics kits are sold and supported by BIOCRATES Life Sciences AG. To purchase kits or for more details about the kit workflow, contact BIOCRATES at sales@biocrates.com. The AB SCIEX mass spectrometers are sold and supported by AB SCIEX. Please contact your local AB SCIEX sales representative or visit www.absciex.com for more information.

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