

### **ThermoFisher** SCIENTIFIC

Find the Targets and Answers that Matter – Introducing the Absolute/DQ p400 HR Kit and Q Exactive MS for Targeted Metabolic and Lipid Profiling



- 2 Required Instrumentation
- **3** Easy and Streamlined Workflow
  - **Components of the All-in-One Solution** 
    - **Analytical Performance**
- 6

4

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**Biological Relevance and Applications** 





## Our Metabolic Phenotype Tells a Story of Who we Are



**BIOCRATES** 

The Deep Phenotyping Company



Health Status

Disease Risk Assessment

Drug and Therapy Response Prediction

Environmental Exposure Precision Medicine Public Healthcare



## Targeted Metabolomics - Quantitation of Key Metabolites in Biological Processes



Thermo Fisher



## Targeted Metabolomics - Quantitation of Key Metabolites During Sleep Deprivation





PNAS | July 22, 2014 | vol. 111 | no. 29 | 10765





## Challenges in Targeted Metabolomics Analysis

The Deep Phenotyping Company





## Absolute *IDQ*<sup>®</sup> p400 HR Kit and Q Exactive Orbitrap MS



- The Absolute IDQ® p400 HR Kit Provides a:
- Standardized
- Quality-controlled and
- Reproducible quantitative assay



The HRAM unique capabilities of the Thermo Scientific<sup>™</sup> Q Exactive<sup>™</sup> Orbitrap MS complements the analysis by providing:

- Resolution and accurate quantitation of isobaric lipid species
- Increased selectivity and specificity for lipid analysis in FIA
- Accurate quantitation with less interferences

Quantitative, Standardized and Reproducible Metabolomics





## Metabolite and Lipid Coverage

### 408 Endogenous Metabolites

- 11 metabolite classes
- > 365 lipids and 43 small molecules
- Amino acids (21)
- Biogenic amines (21)
- Hexoses (1)

#### Polar lipids

- Phosphatidylcholines (172)
- Lysophosphatidylcholines (24)
- Sphingomyelins (31)
- Ceramides (9)

#### Neutral lipids

- Acylcarnitines (55)
- Diglycerides (18)
- Triglycerides (42)
- Cholesteryl esters (14)

### Key Benefits at a Glance

- Ready-to-use quantitative kit for Q Exactive MS platform
- Simple and efficient automated workflow
- High-throughput and minimal sample volume
- Robust platform for longitudinal & inter-lab comparability



### Broad Metabolic and Lipid Profiling





### Instrumentation and Plate Layout

Thermo Scientific<sup>™</sup> Q Exactive<sup>™</sup> Orbitrap<sup>™</sup> Family of HRAM Mass Spectrometers

- ➤ Thermo Scientific<sup>™</sup> Q Exactive<sup>™</sup> Focus MS
- ➤ Thermo Scientific<sup>™</sup> Q Exactive<sup>™</sup> MS
- ➤ Thermo Scientific<sup>™</sup> Q Exactive<sup>™</sup> Plus MS
- ➤ Thermo Scientific<sup>™</sup> Q Exactive<sup>™</sup> HF MS

#### UHPLC

➢ Thermo Scientific<sup>™</sup> Vanquish<sup>™</sup> UHPLC, Thermo Scientific<sup>™</sup> UltiMate<sup>™</sup> 3000 RSLC





Blank and zero samples

Internal standards

Up to 82 samples per plate



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Developed for Q Exactive Orbitrap MS





### Workflow Overview



### Easy and Streamlined Workflow from Sample Preparation to Data Analysis





## Kit Overview

### **Kit Components**

#### Reagents and Consumables

- Patented 96-well filter plates
- Internal standards
- Calibration standards
- Quality controls
- Instrument suitability test mix

#### Methods and Protocols

- Sample preparation protocol
- Instrument-specific acquisition & quantitation methods

#### ➢ Workflow Manager MetIDQ<sup>™</sup>

- Process guidance
- Automated technical validation

### **Benefits**

- All in one box
- Standardized & quality controlled
- Only 10 µL sample required
- Validated for human plasma
- Ready-to-use and user-friendly
- Longitudinal and inter-lab comparability
- Save precious sample material

Standardized and Quality Controlled Assay





## Met/DQ<sup>TM</sup> Software for Workflow Management

#### **Automated Workflow**

#### Information Management

- Sample registration, plate layout design
- Worklist generation

#### Quantitation and Data Processing

- Automated signal identification & quantification
- Data merging

#### Automated Technical Validation

- Transparent display of generated data
- Analytical approval of the plate
- Inter-plate monitoring of QC results and precision

#### Data Evaluation and Export

- Data normalization (optional)
- Export of results in various file formats

#### Statistical Analysis (optional)

Basic statistics & data visualization

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### Streamlined Workflow Management and Data Processing Automation





## Met/DQ<sup>™</sup> Software for Workflow Management

#### **Automated Workflow**

- Information Management
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#### Automated Technical Validation

- Transparent display of generated data
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#### Data Evaluation and Export

- Data normalization (optional)
- Export of results in various file formats
- Statistical Analysis (optional)
  - Basic statistics & data visualization

### **Benefits**



### Transparent, automated and guided process

- Quantitative results for all analytes
- Reduced complexity
- Efficient and time saving
- High confidence in results

Streamlined Workflow Management and Data Processing Automation





### Accuracy Assessed with NIST Reference Material



The field MUST become more quantitative if findings are to be translated to practical, <u>clinical</u> applications."

David Wishart, 2015

### Excellent Accuracies in Measured Concentrations vs the NIST Reference Material





## Analytical Performance: Inter-day Comparability



Excellent Accuracy, Precision and Inter-day Comparability





## Inter-laboratory Comparability



### ➤ 3 laboratories

- 5 instruments (Q Exactive Focus MS and Q Exactive HF MS)
- Reference lab 1 (Q Exactive Focus MS)
- ➢ 6 individual human plasma samples
- Resolution for LC-MS:
- 70k (Q Exactive Focus MS ) 60k (Q Exactive HF MS)

### **Excellent Performance for Amino Acids and Biogenic Amines**





## Inter-laboratory Comparability



#### ➢ 3 laboratories

➢ 6 instruments (Q Exactive Focus MS,

Q Exactive Plus MS and Q Exactive HF MS)

- Reference lab 1 (Q Exactive Focus MS)
- 6 individual human plasma samples
- > Resolution for FIA-MS:

70k (Q Exactive Focus MS ) 70k (Q Exactive Plus MS) 120k (Q Exactive HF MS)

### High Inter-laboratory Comparability for Hexoses, Acylcarnitines and Lipids





## **Analytical Precision**



#### 3 laboratories

- ➢ 6 instruments (Q Exactive Focus MS and Q Exactive HF MS, Thermo Scientific<sup>™</sup> TSQ Vantage<sup>™</sup> MS)
- Reference lab 1 (Q Exactive Focus MS)
- ➢ 6 individual human plasma samples
- ➤ Resolution for LC-MS:

70k (Q Exactive Focus MS ) 60k (Q Exactive HF MS)

### Very Good Analytical Precision in LC-MS for all Q Exactive Orbitrap MS tested





## **Analytical Precision**



#### > 3 laboratories

- 6 instruments (Q Exactive Focus MS
   Q Exactive Plus MS and Q Exactive
   HF MS, Thermo Scientific TSQ
   Vantage)
- Reference lab 1 (Q Exactive Focus MS)
- ➢ 6 individual human plasma samples
- > Resolution for FIA-MS:

70k (Q Exactive Focus MS ) 70k (Q Exactive Plus MS) 120k (Q Exactive HF MS)

### Very Good Analytical Precision in FIA-MS for all Q Exactive Orbitrap MS tested





### Importance of Inter-laboratory Reproducibility

Metabolomics (2016)12:149 DOI 10.1007/s11306-016-1094-6	CrossMark
ORIGINAL ARTICLE	

Metabolomics enables precision medicine: "A White Paper, Community Perspective"

Beger et al., Metabolomics (2016) 12: 149

"[...] Biocrates commercially supplies kits for analysis of targeted areas of metabolism. [...] Each kit provides advantages in interlaboratory use [...]."

### **Ring Trials**

- 2 worldwide first ring trials (2016)
   Readiness of kit technology
- First ring trial scheduled for AbsoluteIDQ p400 HR kit

### Absolute*IDQ* p400 HR kit

- Quantitative results with high accuracy
- Inter-day comparability
- Multi-center and multi-instrument evaluated
- Robust platform
- Confident results





## **Broad Analyte Coverage**



# Membrane composition

**Bionergetic processes (mitochondrial beta** oxidation of fatty acids, glutaminolyis, glycolysis)

> Oncology Cardiology Neurology Aging **Diabetes**

> > .....

#### **Unique Analyte Coverage Provides Important Biological Information**





## Metabolic Signatures-Newborn Screening

Metabolomics (2016)12:149 DOI 10.1007/s11306-016-1094-6	CrossMark				
ORIGINAL ARTICLE					
Metabolomics enables precision medicine: "A White Paper, Community Perspective"					
Metabolomics enables precision medic Community Perspective"	ine: "A White Paper,				

"Today, clinicians capture only a very small part of the information contained in the metabolome, as they routinely measure only a narrow set of blood chemistry analytes ... will be replaced by ... a far more comprehensive metabolic signature."



### **Newborn Screening**

- Phenylketonuria
- Phe ↑, Phe/Tyr > 2.5

- Medium Chain Acyl-Coa-dehydrogenase (MCAD) deficiency
- C6> 0.21, C8> 0.32, C10> 0.48, c10:1> 0.28
- C8/C10 > 1.6, C8/C12> 1.6





## Metabolic Signatures-Precision Medicine

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ORIGINAL ARTICLE					
Metabolomics enables precision medicine: "A White Paper, Community Perspective"					
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"Today, clinicians capture only a very small part of the information contained in the metabolome, as they routinely measure only a narrow set of blood chemistry analytes ... will be replaced by ... a far more comprehensive metabolic signature."

### **Fischer Ratio**

- Important in assessing:
- Liver metabolism
- Liver dysfunction

i.e. BCAA/AAA ratio increases with decrease severity of liver failure



Fischer et al., Am J Surg (1974) 127: 40





## Case Study-Advantage of Broad Metabolite Coverage



- Identification of key metabolites for ovarian cancer
- > AA and AC are the top markers
- Importance of affected pathways in tumor pathophysiology and other disease
- Unique metabolite portfolio of the AbsoluteIDQ<sup>®</sup> p400 HR Kit provides added value

Biocrates Life Sciences in collaboration with Prof. Burwinkel (Heidelberg University, Germany)

Identification of Key Metabolites that are Significantly Altered Between the two Groups





### Conclusions

- The Absolute IDQ<sup>®</sup> p400 HR Kit is a ready-to-use solution designed for targeted metabolomics on Q Exactive Orbitrap HRAM MS.
  - Ready to implement within a day, without method development
- The kit provides quantitative results for up to 408 metabolites covering a broad range of biological functions.
- The kit delivers a standardized, quality-control, and targeted quantitative assay for accurate and confident metabolic profiling
- The simple, efficient, and automated workflow ensures high throughput and use of minimal sample volume.
- The robust platform with standardized procedures assure longitudinal and inter-lab comparability.

Standardized and Reproducible Quantitation of Hundreds of Lipids and Small Molecules for Broad Lipid and Metabolic Profiling





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