1.1 Quantitative Bioanalytical Methods

Quantitative Bioanalytical Methods Compounds

Compound	Matrix	Lower Limit of Quantitation	Instrument
ADMA, Homocysteine, Arg	Plasma	0.3 µM	API 4000
Alfentanil	Plasma	0.1 ng/mL	API 5000
Aprotinin	Tissue (Rat Kidney)	80 ng/mL	ELISA
Biolimus	Blood, Tissue, Stents	0.005 ng/mL	API 5000
Cyclosporine / Metabolites	Blood	0.1-1.0 ng/mL	API 5000
DMXB	Plasma/Brains	0.1 ng/mL	API 5000
Duet DNA	Monocytes	Ratio	GC-MS
Everolimus	Blood	0.1 ng/mL	API 5000
Felbamate	CSF, Brain, Serum	0.1 ng/mL	API 5000
Fentanyl	Plasma/DBS	0.1 ng/mL	API 4000
Free-Fatty Acids	Plasma, Tissue, Blood	100 µM	GC-MS
GSH	Plasma	10 µM	API 4000
Glucocorticiods	Plasma	0.1 ng/mL	API 5000
[^{13}C] Glucose	Plasma	10 µM	GC-MS
[^{13}C] Glycerol	Plasma	10 µM	GC-MS
High Energy Phosphates	Tissues	0.25 µM	API 4000
Isoprostanes	Plasma, Urine	0.01 ng/mL	API 5000
Ketamine	Blood	1.0 ng/mL	UPLC-MS/MS
Ketarolac	Plasma	1.0 ng/mL	API 4000
Lamotrigine	Plasma	1.0 ng/mL	API 4000
Leflunomide	Blood	0.1 ng/mL	API 5000
Lidocaine	Plasma	0.5 ng/mL	API 4000
Lovastatin	Plasma	0.1 ng/mL	API 5000
Metabolic Profiling	Plasma, Urine, Tissue	_____	Exactive
Morphine / Metabolites	Plasma/DBS	1 - 2.5 ng/mL	API 5000
MPA	Plasma	1.0 ng/mL	API 4000
Naltrexone	Plasma	0.1 ng/mL	API 5000
Nicotine	Hair	0.1 ng/mL	API 5000

Quantitative Bioanalytical Methods

Compounds

Compound	Matrix	Lower Limit of Quantitation	Instrument
Pravastatin	Plasma	0.5 ng/mL	API 4000
Phenytoin	Plasma	0.1 ng/mL	API 5000
Phenytoin	Plasma	0.1 ng/mL	API 5000
PhIP	Plasma / Microsomes	0.1 ng/mL	API 4000
Propofol	Plasma	0.5 ng/mL	API 4000
Sirolimus	Tissues / Blood	0.01 ng/mL	API 4000
Steroid Hormones	Plasma	0.1 ng/mL	API 5000
Tacrolimus / Metabolites	Blood	0.1 ng/mL	API 4000
Temsirolimus / Metabolites	Blood	0.1 ng/mL	Exactive
Valproic Acid	Serum	1.0 ng/mL	GC/MS
Vitamin D and Metabolites	Plasma	0.1 ng/mL	API 5000
Paclitaxel	Blood	0.25ng/mL	API 5000
Methadone	Plasma/DBS	0.25ng/mL	API 4000

Quantitative Bioanalytical Methods Amino Acids

Amino Acids	Matrix	Lower Limit of Quantitation	Instrument
Ala-Gin	Plasma	20nM/mL	API 5000
Alanine	Plasma	20nM/mL	API 5000
Arginine	Plasma	20nM/mL	API 5000
Asparagine	Plasma	20nM/mL	API 5000
Aspartic Acid	Plasma	20nM/mL	API 5000
(Cysteine) 2	Plasma	20nM/mL	API 5000
Citruline	Plasma	20nM/mL	API 5000
Glutamic Acid	Plasma	20nM/mL	API 5000
Glutamine	Plasma	20nM/mL	API 5000
Glycine	Plasma	20nM/mL	API 5000
Gly-Gin	Plasma	20nM/mL	API 5000
Histidine	Plasma	20nM/mL	API 5000
Hydroxyproline	Plasma	20nM/mL	API 5000
Isoleucine	Plasma	20nM/mL	API 5000
Lysine	Plasma	20nM/mL	API 5000
Mehtionine	Plasma	20nM/mL	API 5000
Mehtionine–d3*	Plasma	20nM/mL	API 5000
Ornithine	Plasma	20nM/mL	API 5000
Phenylalanine	Plasma	20nM/mL	API 5000
Proline	Plasma	20nM/mL	API 5000
Threonine	Plasma	20nM/mL	API 5000
Tryptophan	Plasma	20nM/mL	API 5000
Tryptohan-d5*	Plasma	20nM/mL	API 5000
Tyrosine	Plasma	_20nM/mL	API 5000

1.2 LC-MS/MS Quantification of Endogenous Compounds

Free Isoprostanes
Method : LC/LC-MS/MS
Status : fully validated
Matrices : human plasma, human urine, rat plasma and urine, tissue (partially validated)
Reference : Haschke M, Zhang YL, Kahle C, Klawitter J, Korecka M, Shaw LM, Christians U. Quantification of 15-F2t-isoprostane in human urine and plasma using high-performance liquid chromatography - atmospheric pressure chemical ionization-tandem mass spectrometry. Clin Chem 2007; 53:489-497.

Total Isoprostanes
Method : LC/LC-MS/MS
Status : partially validated
Matrices : human plasma, human urine, rat plasma and urine
Reference : N/A. Modification of the free isoprostane assay including release of bound isoprostanes using KOH human plasma, human urine, rat plasma and urine and extraction with affinity columns.

Vitamin D Profiling
VitD2, VitD3, 25(OH)VitD2, 25(OH)VitD3, 1(OH)VitD3, 1,25(OH)2VitD2 and 1, 25(OH)2VitD3
Method : LC/LC-MS/MS
Status : partially validated
Matrices : human plasma
Reference : ASMS presentation, publication in preparation

Steroid Hormones
Testosterone (total and free), estrogen (total)
Method : LC/LC-MS/MS
Status : partially validated
Matrices : human plasma
Reference : publication in preparation

In Preparation:
Endothelin, angiotensin, aldosterone

Endothelial Dysfunction Panel (LC-MS/MS)

- ADMA
- Arginine
- Homocysteine
- Cysteine
- Glutathione
- Isoprostanes
- Amino acid profiles in plasma
- Fatty acid profiles in blood cells
- HODE/ HETE (bioactive lipids)

1.3 Amino Acids

Method : LC-MALDI-TOF/MS
Status : fully validated
Matrices : human plasma
Reference : Armstrong M, Jonscher K, Reisdorph NA. Analysis of 25 underivatized amino acids in human plasma using ion-pairing reversed-phase liquid chromatography/time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 2007;21(16):2717-26.

Analytes:

Compound	Molecular Formula	Exact mass [M+H]	Extracted ion window	Low cal std (nM/mL)	High cal std (nM/mL)	S/N ratio (pM injected)	IS used for Quantitation
Taurine	C2H7N03S	126.0224	126.00-126.04	1.56	400	851 (125)	Glutamine-d5
Aspartic acid	C4H7N04	134.0453	134.01-134.05	1.56	400	52.5 (125)	Glutamine acid-d3
Hydroxyproline	C5H9N03	132.0660	132.03-132.07	1.56	400	1750 (125)	Glutamine-d5
Serine	C3H7NO3	106.0504	106.02-106.06	1.56	400	267 (125)	Glutamine acid-d3
Glycine	C2H5NO2	76.0398	76.01-76.05	25	3200	22.3 (3125)	Glutamine acid-d3
Glutamine-d*	C5H5D5N203	152.1000	152.05-152.15	NA	NA	637 (1000)	NA
Glutamine	C5H10N2O3	146.0769	147.04-147.08	25	3200	1450 (3125)	Glutamine-d5
Asparagine	C4H8N2O3	133.0613	133.03-133.07	1.56	400	94.9 (125)	Glutamine-d5
Threonine	C4H9NO3	120.0660	120.03-120.07	1.56	400	315 (125)	Glutamine acid-d3
Glutamic acid-d*	C5H6D3NO4	151.1000	151.05-151.15	NA	NA	15.2 (1000)	NA
Glutamic acid	C5H9NO4	148.0609	148.03-148.07	12.5	1600	714 (1562)	Glutamine acid-d3
Alanine	C3H7N02	90.0555	90.02-90.06	12.5	1600	345 (125)	Leucine-d10

Compound	Molecular Formula	Exact mass [M+H]	Extracted ion window	Low cal std (nM/mL)	High cal std (nM/mL)	S/N ratio (pM injected)	IS used for Quantitation
(Cysteine)2	C6H12N2O4S2	241.0316	241.01-241.05	1.56	400	1160 (125)	Methionine-d3
Citrulline	C6H13N3O3	176.1035	176.01-176.05	1.56	400	383 (125)	Glutamine-d5
Proline	C5H9NO2	116.0711	116.04-116.08	1.56	400	355 (125)	Glutamine-d5
Gly-Gln*	C7H13N3O4	204.2000	204.10-204.30	NA	NA	2560 (1000)	NA
Ala-Gln	C8H15N3O4	218.1140	218.08-218.12	1.56	400	508 (125)	Gly-Gln
Valine	C5H11NO2	118.0868	118.05-118.09	1.56	400	163 (125)	Leucine-d0
Methionine-d3*	C5H8DNO2S	153.1000	153.05-153.15	NA	NA	1810 (1000)	NA
Methionine	C5H11NO2S	150.0588	150.03-150.07	1.56	400	737 (125)	Methionine-d3
Tyrosine	C9H11NO3	182.0817	182.05-182.09	1.56	400	722 (125)	Leucine-d10
Isoleucine	C6H13NO2	132.1024	132.08-132.12	1.56	400	340 (125)	Leucine-d10
Leucine-d10*	C6H3D10NO2	142.2000	142.00-142.30	NA	NA	1450 (1000)	NA
Leucine	C6H13NO2	132.1024	132.08-132.12	1.56	400	228 (125)	Leucine-d10
Phenylalanine	C9H11NO2	166.0868	166.06-166.10	1.56	400	1010 (125)	Leucine-d10
Histidine	C6H9N3O2	156.0773	156.05-156.08	1.56	400	1090 (125)	Tryptophan-d5
Tryptophan	C11H12N2O2	205.0977	205.08-205.12	1.56	400	383 (125)	Tryptophan-d5
Tryptophan-d5*	C11H7D5N2O2	210.1000	210.00-210.30	NA	NA	1110 (1000)	NA
Arginine	C6H14N4O2	175.1195	175.09-175.13	1.56	400	1700 (125)	Tryptophan-d5
Ornithine	C6H12N2O2	133.0977	133.08-133.12	1.56	400	544 (125)	Tryptophan-d5
Lysine	C6H14N2O2	147.1133	147.09-147.13	1.56	400	448 (125)	Tryptophan-d5

* Internal standard

1.4 High Energy Phosphates (LC-MS)

Method : LC-MS
Status : fully validated
Matrices : Tissues
Reference : Klawitter J, Schmitz V, Klawitter J, Leibfritz D, Christians U. Development and validation of an assay for the quantification of 11 nucleotides using LC/LC-electrospray ionization-MS. Analytes Biochem. 2007 Jun15;365(2):230-9.

Analytes:

AMP (m/z= 346)

ADP (m/z= 426)

ATP (m/z= 506)

GDP (m/z= 442)

GTP (m/z= 522)

UDP (m/z= 403)

UTP (m/z= 483)

CDP (m/z= 402)

CTP (m/z= 482)

NAD (m/z= 662)

FAD (m/z= 784)

Internal Standard 6-aminohexyl-ADP (m/z= 525

1.5 Metabolic Profiling (GC-MS and LC-MS)

Method : GC-MS
Status : partially validated
Matrices : human and rat plasma and tissues
Reference : N/A : N/A

Analytes:

3OH-butyrate
Acetic acid
Alanine
Aminomalonate
Creatinine
Galactonic acid
Glucitol
Glucoronic acid
Glutamine
Glycine
Hippurate
Histidine
Indolacetate
Isocitrate
Isovalerate
Lactic acid
Oxalate
Proline
Pseudouridine
Pyruvic acid
Ribose
Serine
Succinate
Threonine
Tyrosine
Uric acid
Valine
Xylitol

Please note that only the major metabolites are listed. Typically, more than 100 metabolites can be detected within one GC-MS run, allowing either for quantitation or semi-quantitative comparison.

In addition, LC-TOF and LC-MS-TOF assays are set up and available. Metabolites can be identified using the METLIN / HMBD databases. However, it must be noted that due to the inherent problem of ion suppression in the electrospray source use of this data for semi-quantitative comparison may be limited.

1.6 Metabolic Profiling (^{1}H NMR)

Method : proton nuclear magnetic resonance spectroscopy (500, 600 and 900 MHz)
Status : partially validated
Matrices : human and rat plasma, urine and tissues
Reference : N/A

Analytes:

No.	Metabolite	1H [ppm]	13C [ppm]	blood	urine
1	Leucine	0.94	22	●	●
2	Leucine	0.96	23	●	●
3	Valine	0.97	18	●	
4	Isoleucine	1.00	16	●	●
5	Valine	1.03	19	●	
6	(isobutyrate / 3oxo-isovalerate)	1.13	23.1		●
7	3-hydroxybutyrate	1.19	23	●	
8	Threonine	1.29	20.5	●	
9	Lactate	1.32	21.3	●	●
10	Lysine	1.43 + 1.47	22.8	●	
11	Alanine	1.46	17.5	●	●
12	Arginine	1.67	25.3	●	
13	Leucine	1.68	25.0	●	●
14	Leucine	1.69	41.0	●	
15	Leucine	1.69	40.2	●	
16	Lysine	1.70	27.6	●	
17	2-hydroxyglutarate	1.83	31.9		●
18	Lysine	1.87	31.5	●	

No.	Metabolite	1H [ppm]	13C [ppm]	blood	urine
19	Arginine	1.87	29.3	●	
20	2-Hydroxyglutarate	1.98	31.5		
21	Acetate	2.02	23.4	●	●
22	Glutamate	2.08	28.3	●	
23	Glutamine	2.12	28	●	
24	Hydroxyglutarate	2.26	34.3		●
25	Glutamate	2.32	34.7	●	
26	Succinate	2.43	34		●
27	Glutamine	2.44	32.1	●	
28	2-Oxoglutarate	2.45	31.5		●
29	Glutathione (Glu) both froms	2.49	32.7	●	
30	Citrate	2.56	46.2		●
31	Dimethylamine	2.70	35.7		●
32	Citrate	2.71	46.2		●
33	Trimethylamine	2.87	45.7		●
34	GSSG (Cys) oxidized	2.95	39.9	●	
35	2-Oxoglutarate	2.97	36.8		●
36	Lysine	3.01	40.3	●	
37	Creatine	3.02	38.3	●	●
38	Creatinine	3.04	31.4	●	●
39	R-N+-$(CH_3)_3$ several signals	3.18 -3.27	53.1-55.5	●	●
40	Taurine	3.20	49.9	●	●
41	β-Glucose C2	3.23	75.3	●	●
42	Arginine	3.23	41.7	●	
43	Trimethylamine N-oxide	3.25	60.8	●	● 3.3 ppm

No.	Metabolite	1H [ppm]	13C [ppm]	blood	urine
44	Phenylalanine	3.28	36.9		●
45	GSSG (Cys) oxidized	3.30	39.8	●	
46	Taurine	3.32	36.8	●	●
47	α, β-Glucose C4	3.40	70.8	●	●
48	β-Glucose C3/C5	3.48	77.1	●	●
49	α-Glucose C2	3.52	72.6	●	●
50	Glycine	3.54	42.8	●	●
51	Glutathione+Gln+Glu	3.69	55.7	●	
52	α-Glucose C3	3.69	73.9	●	●
53	α, β-Gglucose C6	3.72	61.9	●	●
54	Glutathione (Gly) both forms	3.75	44.7	●	
55	Alanine	3.75	51.7	●	●
56	α-Glucose C5	3.82	72.6	●	●
57	α, β-Glucose C6	3.87	61.9	●	●
58	Creatine	3.91	55.0	●	●
59	Hippurate	3.94	45.1		●
60	Creatinine	4.09	56.9		●
61	Lactate	4.09	69.7	●	●
62	β-Glucose C1	4.64	97.1	●	●
63	α-Glucose C1	5.21	93.3	●	●
64	Urea	5.80	------		●
65	Phenylalanine	7.31	130.5		●
66	Phenylalanine	7.34	128.0		●
67	Phenylalanine	7.41	129.9		●
68	Hippurate	7.50	129.9		●

1.6 Metabolic Profiling (^{1}H NMR)

No.	Metabolite	1H [ppm]	13C [ppm]	blood	urine
69	Hippurate	7.59	133.2		●
70	Hippurate	7.79	128.2		●
71	Fumarate	8.46	147.9		

1.7 High Energy Phosphates (^{31}P NMR)

Method : phosphorous nuclear magnetic resonance spectroscopy
Status : partially validated
Matrices : Tissues
Reference : N/A

Analytes:

- phosphomonoesthers (PME)
- phosphodiesters (PDE) (both precursors for membrane phospholipid metabolism)
- sugar phosphates (UDPG) phosphocreatine (PCr)
- NAD,
- NMP
- NDP
- NTP
- Anorganic phosphate (can be used for monitoring in vivo pH changes in perfused organs and cells)
- NMP, NDP and NTP: nucleotide mono, di and tri phosphates such as AMP, ADP, ATP etc.

Lipidomics can be defined as the comprehensive identification and quantification of all lipid molecular species in a biological system. Lipids are loosely defined as biological compounds that are generally hydrophobic in nature and soluble in organic solvents. Lipids serve as membrane components, as mediators in cell signaling and as fuel and energy storage and are of interest in areas such as cardiovascular disease, cancer, inflammation, and nutrition. Their distinct solubility properties often dictate their separate analysis in metabolomics experiments. iC42 assays for lipid profiling are based on ^{1}H-NMR, GC-MS and LC-MS/MS and, as aforementioned, include non-targeted screening as well as targeted, validated LC-MS/MS assays for bioactive lipids, isoprostanes, prostaglandins and leukotrienes.

1.8 Fatty Acid Profiling (GC-MS)

Method : GC-MS
Status : partially validated
Matrices : human and rat plasma and tissues
Reference : N/A

Analytes:

C12:0 dodecanoic acid (lauric acid)

C14:0 tetradecanoic acid (myristic acid)

C14:1 tetradecenoic acid (myristoleic acid)

C16:0 hexadecanoic acid (palmitic acid)

C16:1 hexadecenoic acid (palmitoleic acid)

C17:0 heptadecanoic acid (margaric acid) as internal standard

C18:0 octadecanoic acid (stearic acid)

C18:1 octadecenoic acid (oleic acid)

C18:2 octadecadienoic acid (linoleic acid)

C18:3 octadecatrienoic acid (linolenic acid)

C20:4 eicosatetraenoic acid (arachidonic acid)

C22:0 docosanoic acid (behenic acid)

C24:0 tetracosanoic acid (lignoceric acid)

1.9 Lipid Patterns (^{1}H NMR)

Method : proton nuclear magnetic resonance spectroscopy
Status : human and rat blood, plasma and tissues
Matrices : human and rat blood, plasma and tissues
Reference : N/A

Analytes:

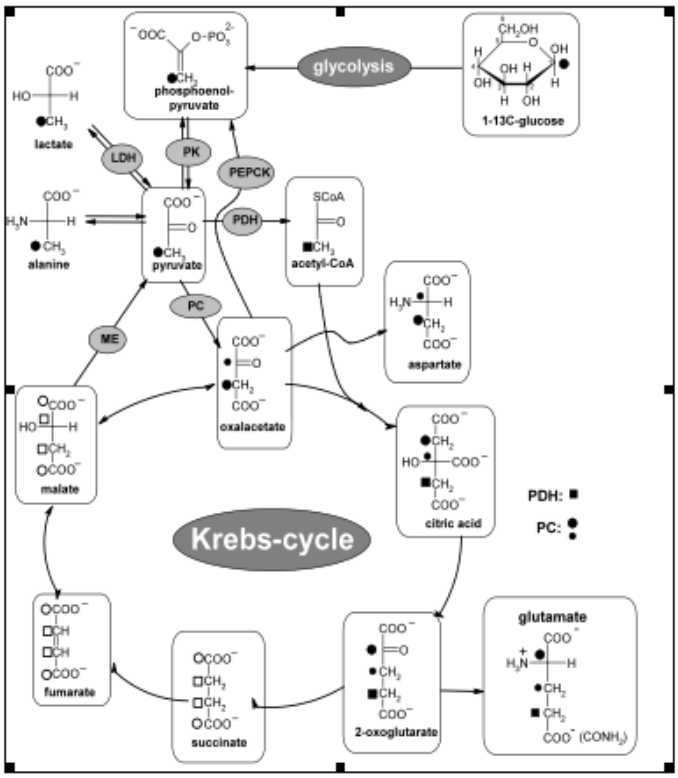
- poly- and mono-unsaturated fatty acids (PUFA and MUFA)
- total fatty acids
- triacylglycerols
- glycerophosphates
- phosphatidylcholine
- phosphatidylethanolamine
- cholesterol

1.10 ^{13}C-labeled tracers (^{13}C NMR)

A powerful non-biased, non-targeted screening metabolomics tool to identify unknown molecular mechanisms, is the assessment of fluxes in the metabolic network of a cell, organ, or organism: **Fluxomics**. By capturing the metabolome in its functional interactions with the environment and the genome, it provides a true dynamic picture of the phenotype. The most reliable strategy to do this is *via* ^{13}C-labeled substrates: the ^{13}C atoms are incorporated into the newly formed downstream metabolites in distinct numbers and specific positions allowing identification of distinct isotopomers and the evaluation of metabolite fluxes through specific pathways and the changes hereof as caused by drugs and disease processes.

Method	^{13}C nuclear magnetic resonance spectroscopy
Status	partially validated
Matrix	tissues, *ex vivo* perfused organs, perfused and extracted cell cultures
Reference	N/A

Analytes: Depending on ^{13}C labeled tracer used. For example in the case of 1-^{13}C-glucose:



Metabolic fate of ^{13}C-label from [1–^{13}C]glucose. Label distribution in glycolytic and tricarboxylic acid (TCA) cycle intermediates during metabolism of [1–^{13}C]glucose

1.11 Isotope Ratio Mass Spectrometry and Measurement of Isotope Enrichment Using GC-MS

Carbon Dioxide Enrichment

Method : Automated carousel and reference gas system in combination with a double-focusing sector field mass spectrometer
Status : partially validated
Matrices : breath and blood
Reference : N/A

Singly Labeled Water for Determination of Total Body Water and Doubly Labeled Water Enrichment for the Measurement of Total Energy Expenditure

Method : double-focusing sector field mass spectrometer with autosampler, an HD collector, dual inlet system, and an H/device for reduction of H_2O to H_2
Status : partially validated
Matrices : human and rat plasma
Reference : N/A

Labeled Glucose and Glycerol in Plasma

Method : GC-MS
Status : fully validated
Matrices : human and rat plasma
Reference : N/A

The assay was developed and validated using 6,6-d2-glucose and d5 glycerol. This method can be easily adapted to glucose and glycerol with other ^{13}C and deuterium labeled analogues.

Labeled Essential and Non-Essential Amino Acids

Method : GC-MS
Status : fully validated
Matrices : human and rat plasma
Reference : N/A

1-^{13}C-labeled Fatty Acids

Method : GC-MS
Status : fully validated
Matrices : human and rat plasma
Reference : N/A

1.12 Non-Targeted Proteomics

Extraction, Separation and Analytical Technologies:

- 1D and 2D-gel electrophoresis
- Spot cutting, trypsination
- Immunoprecipitation
- Fractionation by semi-preparative HPLC
- 1D- and 2D HPLC, nano-LC, chromatography chip
- MALDI
- Protein identification: iontrap-MS spectrometry, quadrupole-time-of-flight mass spectrometry, triple stage quadrupole mass spectrometry, quadrupole- linear ion trap mass spectrometry in combination with nano-LC and database searches.

Labeling Technologies:

- SILAC (in combination with cell culture facility)
- iTRAQ

Qualification of Database Hits:

- Western blot
- PCR
- gene knock down (in combination with cell culture facility)

Proteomics Services

From sample preparation to generating high quality data for publication, in the following areas:

- Proprietary sample preparation technology
- 2D DIGE (2-Dimensional Differential In-Gel Electrophoresis) for detection of differential protein expression
- 2D Phosphoprotein and Glycoprotein profiling
- Protein identification by Mass Spectrometry
- 2D membrane service and fluorescent 2-D Western Blot
- Serum Proteomics

Our dedicated service team and cutting-edge technology platforms offer the following:

- Customized study design and sample preparation

- High sensitivity: 0.2ng / spot
- High accuracy: in-gel analysis of up to 3 samples & cross-gel analysis of > 3 samples
- Fast turnaround time: one week
- Cost-effectiveness: price covers experimental design & sample preparation
- High-quality data: ready for publication and presentation