Metabolomics Core Technology Platform
University of Heidelberg

General guideline for targeted and untargeted metabolite analyses:

- As billing is done shortly after sample analyses, please inform us as soon as possible if you plan to analyze additional samples. Additional samples need to be submitted at the latest two weeks after the recent analysis to qualify for a quantity discount. Therefore we also ask you to inform us in advance about the total number of samples you plan to examine.

- After the final data as well as the original data files have been handed over to the user, the data will be stored by the MCTP in copy for two months. Any remaining samples get discarded two weeks after data have been sent to the user. Please inform us beforehand if you intend to collect remaining sample material.

- Controls and blank samples also count as samples and need to be listed during service request and on any documents.

Sample submission:

- **For the analyses of liquid samples**, please provide an adequate amount (see list in the end) of pure medium together with your samples which can be used as "blank" or for spiking experiments.
  Furthermore, if your sample or standard substances are not dissolved in water, inform the MCTP about the pH of the solution and the medium composition (e.g. salts) as this might significantly alter retention time, baseline or signal strength (matrix effects).
  We kindly ask you to filtrate all liquid samples prior submission using 0.2µm filters.
  Please hand in a sheet/email with respective reference values of the samples (e.g. volume, solvent etc.).

- **For the analysis of solid samples**, please provide an appropriate amount of sample. A list with minimum quantity you can find in the end of this document. Please deliver all samples without remaining supernatant. Solid samples must be previously grounded by use of e.g. mortars or ball mills. Take care that the samples remain deep-frozen at all times during grinding (use liquid nitrogen). Please hand in a Excel or Word file with respective reference values of the samples (e.g. dry weight, fresh weight, number of cells, etc.).

- All samples need to be delivered in suitable vessels. Please refer to detailed information below.

- **Please label your samples (Falcon or Eppendorf tubes) with consecutive numbers and your initials only.**
  If this is not followed, an additional charge of 10% may be added to the total price for sorting and identification of samples.
  Nevertheless, we are pleased to match final results with further sample information if an Excel or Word file is provided.

- For labeling, we kindly ask you to use permanent markers. **Please do not use any adhesive labels/tags as they won't stick to the tubes anymore upon freezing at -80°C or in liquid nitrogen.**

- During transport, samples must remain deep-frozen at any time. Therefore, plus bring your samples on dry ice or in liquid nitrogen.

Method development for targeted analyses:

- For absolute metabolite quantifications (beside the analyses stated on the MCTP homepage), we request the user to provide an aliquot of the pure analyte(s) and its molecular weight.
  Upon agreement with the customer, purchases of chemicals can also be done by the MCTP at the expense of the user.

- If available, please inform us about the approximate concentration of the metabolites of interest in your sample. This will significantly advance method development and preparation of standards.

- Please provide us with any information about the detection/separation of the metabolites of interests that are known to you.

- Development and establishment of new methods and measurement of test samples needs to be charged according to expenditure of time and chemicals/materials used. Determination of recovery rate of metabolites in different matrices is not carried out by default and will only be done upon request due to high time consumption and needs to be charged in addition.

- Method validation: Validation of analytical methods is in general only important for pharmaceutical analysis when ensurance of the continuing efficancy and safety of each batch manufactured relies solely on the determination of quality. The ability to control this quality is dependent upon the ability of the analytical methods, as applied under well-defined conditions and at an established level of sensitivity, to give a reliable demonstration of all deviation from target criteria.

- Method development and validation can be simultaneous, but they are two different processes, both downstream of method selection. Therefore, method validation is only performed upon assignment and associated with additional significant costs – due to protocols set out in the International Conference on Harmonization (ICH) guidelines The US Food and Drug Administration (FDA) and US Pharmacopoeia (USP) both refer to ICH guidelines. The most widely applied validation characteristics are accuracy, precision (repeatability and intermediate precision), specificity, detection limit, quantitation limit, linearity, range, robustness and stability of analytical solutions.
  For scientific publications of metabolite data sets it is normally NOT necessary to use fully validated methods.

Heidelberg, October 2016

Required sample quantities and containers

CELL PELLETS specific Part/Tissue	Analysis	Optimal amount (minimal amount) *10^6	Container
E.g. Blood cells, Murine cells, Human cell lines, Cancer cells	Adenosines	**1,5** (0,75)	1.5ml Tube "Safe Seal"
	AA + Polyamines	**1** (0,5)	
	Anions	**3** (2)	1.5ml Screw top tube (heat-resistant)
	α-Ketoacids	**1,5** (0,75)	1.5ml Tube "Safe Seal"
	Cations	**3-4** (2)	1.5ml Screw top tube (heat-resistant)
	Hexoses	**2** (1)	1.5ml Tube "Safe Seal"
	Nucleotides	**5** (3)	
	TCA cycle compounds	**3** (2)	
	Thiols	**3** (2)	
	Tryptophan and Trp-related metabolites	**3** (2)	
	semi-targeted (GCMS)	**6** (4)	
Primary cells	Adenosines	**2** (1)	
	AA + Polyamines	**2** (1)	
	α-Ketoacids	**3** (2)	
	Nucleotides	**5**	
	TCA cycle compounds	**3** (2)	
	semi-targeted (GCMS)	**8** (6)	

Note!:

- Each analysis requires a separate aliquot.

Exception: Sample material for the analysis of Adenosines, Amino acids, Polyamines and Thiols can be handed over in one tube.

- Please carefully remove any supernatant completely before freezing.

LIQUID SAMPLES	Analysis	Optimal amount (minimal amount) µl	Container
E.g. cellculture supernatants, bacterial supernatants, blood plasma, sera	Adenosines	**250** (100)	1.5ml Tube "Safe Seal"
	AA + Polyamines	**100** (25)	
	Anions	**70** (35)	1.5ml Screw top tube (heat-resistant)
	α-Ketoacids	**200** (60)	1.5ml Tube "Safe Seal"
	Hexoses	**200** (100)	1.5ml Screw top tube (heat-resistant)
	Nucleotides	**100** (25)	1.5ml Tube "Safe Seal"
	TCA cycle compounds	**150** (30)	
	Thiols	**150** (30)	
	Tryptophan and Trp-related metabolites	**400** (200)	
	Urea	**350** (150)	
	Total fatty acids (TFA)	**100** (50)	
	semi-targeted (GCMS)	**200** (100)	

Note!:

- Liquid samples do not need to be aliquoted beforehand.
- TFA analysis and GCMS profiling can be performed from the same sample

MAMMALS	Analysis	Optimal amount (minimal amount)	Container
specific Part/Tissue		mg	
E.g. liver, kidney, heart, spleen, brain, lung, muscle, tumor tissue	Adenosines	25 (10)	1.5ml Tube "Safe Seal"
	AA + Polyamines	25 (10)	
	Anions	25 (10)	1.5ml Screw top tube (heat-resistant)
	α-Ketoacids	30 (20)	1.5ml Tube "Safe Seal"
	Carbohydrates	40 (20)	1.5ml Screw top tube (heat-resistant)
	Nucleotides	25 (10)	1.5ml Tube "Safe Seal"
	TCA cycle compounds	25 (10)	
	Thiols	25 (10)	
	Total fatty acids (TFA)	50 (25)	
	semi-targeted (GCMS)	100 (50)	

Note!:

- Each analysis requires a separate aliquot.

Exception: Solid material for the analysis of Adenosines, Amino acids, Polyamines and Thiols can be handed over in one tube.

- TFA analysis and GCMS profiling can be performed from the same sample.
- Sample material has to be grinded before submission.

PLANTS	Analysis	Optimal amount (minimal amount)	Container
specific Part/Tissue		mg	
E.g. seeds, seedlings, leaves, stems, roots	Adenosines	25 (10)	1.5ml Tube "Safe Seal"
	AA + Polyamines	25 (10)	
	Anions	40 (20)	1.5ml Screw top tube (heat-resistant)
	α-Ketoacids	25 (10)	1.5ml Tube "Safe Seal"
	Cations	30 (15)	1.5ml Screw top tube (heat-resistant)
	Flavonoids	30 (15)	1.5ml Tube "Safe Seal"
	Carbohydrates	40 (20)	1.5ml Screw top tube (heat-resistant)
	TCA cycle compounds	40 (20)	
	Thiols	25 (10)	
	Total fatty acids (TFA)	50 (25)	
	semi-targeted (GCMS)	100 (50)	1.5ml Tube "Safe Seal"

Note!:

-Each analysis requires a separate aliquot.

Exception: Solid material for the analysis of Adenosines, Amino acids, Polyamines and Thiols can be handed over in one tube.

- TFA analysis and GCMS profiling can be performed from the same sample.
- Sample material has to be grinded before submission.

DROSOPHILA	Analysis	Optimal amount (minimal amount)		Container
specific Part/Tissue		µl	Number	
Whole fly	Adenosines		**5** (3)	1.5ml Tube "Safe Seal"
	AA + Polyamines		**5** (3)	
	semi-targeted (GCMS)		**50** (30)	
Hemolymphe	Hexoses	**10** (3)		1.5ml Screw top tube (heat-resistant)
	TCA cycle compounds	**20** (10)		1.5ml Tube "Safe Seal"

Note!:

- Each analysis requires a separate aliquot.

Exception: Solid material for the analysis of Adenosines, Amino acids, Polyamines and Thiols can be handed over in one tube.

YEAST	Analysis	Optimal amount (minimal amount)	Container
		OD cells	
	Adenosines	**20** (10)	1.5ml Tube "Safe Seal"
	AA + Polyamines	**10** (5)	
	Thiols	**10** (5)	
	Total fatty acids (TFA)	**20** (10)	
	semi-targeted (GCMS)	**20** (10)	

Note!:

- Each analysis requires a separate aliquot.

Exception: Solid material for the analysis of Adenosines, Amino acids and Thiols can be handed over in one tube.

- TFA analysis and GCMS profiling can be performed from the same sample.

XENOPUS	Analysis	Optimal amount (minimal amount)	Container
specific Part/Tissue		number	
Oocytes	α-Ketoacids	**20** (10)	1.5ml Tube "Safe Seal"
	semi-targeted (GCMS)	**20** (10)	
Embryo	Adenosines	**10** (4)	
	Nucleotides	**10** (5)	
	TCA cycle compounds	**10** (5)	

Note!:

- Each analysis requires a separate aliquot.