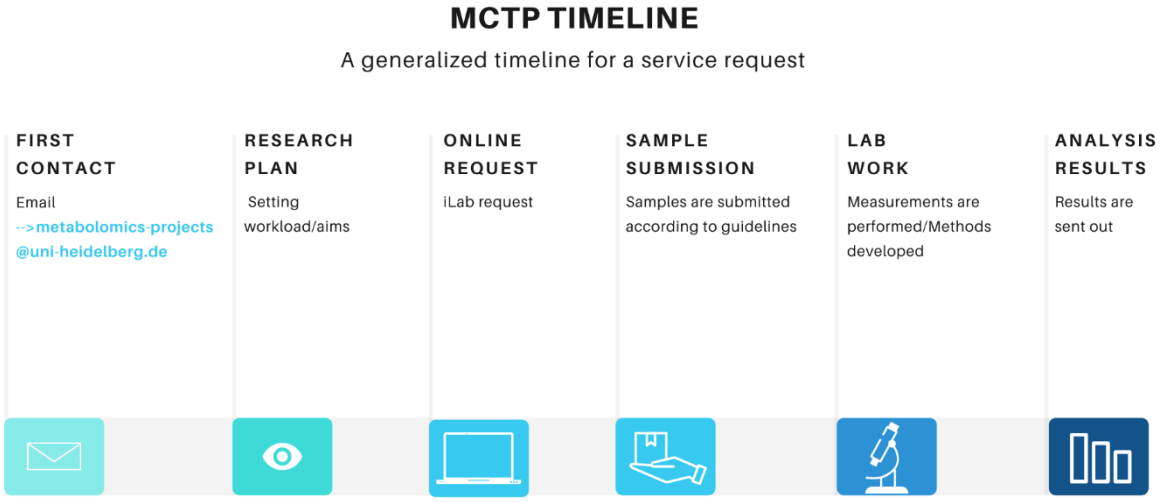
User Guidelines for Metabolite Analyses

The Metabolomics Core Technology Platform (MCTP) of Heidelberg University provides different targeted/untargeted metabolite analyses. Besides our established methods that can directly be requested via our online booking system, we also develop and establish new methods upon request. For the latter, please reach out to us to discuss and plan your research question.



Contact Details

Project/Method related inquiries: **metabolomics-projects@uni-heidelberg.de**

Sample submission related inquiries: **metabolomics-samples@uni-heidelberg.de**

Direct request submission via **online booking system**:

https://hmls.corefacilities.org/landing/1492

Sample Submission Guidelines

We kindly ask you to adhere to the following guidelines:

- Prior to submission request our services via the online booking system iLab.
    - Please put forward a request per method if the same sample should be used for different methods.

- Provide samples **clearly labelled** on the lid.
    - Please do not use any adhesive labels/tags as they won't stick to tubes anymore upon freezing at -80°C or in liquid nitrogen.
    - Use permanent markers.

- Use the following simple labeling code:
  Your initials + consecutive number only, e.g. Max Mustermann: MM_01
    - If this is not followed, an additional charge of 10% will be added to the total price for sorting and identification of samples.
    - Provide additional information via your iLab request, such as normalization, control vs treatment etc.

- **The minimal number of samples per service request is 10 samples**. We do not accept project requests with a lower number of samples.
  Exceptions are samples for testing and method setup if this has been discussed with MCTP staff prior to submission.

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

- Please provide an appropriate amount of sample, as well as standardized sample quantities (equal material per tube/well/plate). → see list below.

- During sample submission the following information is required for each sample:
  "**Label on Tube**" - "**Amount of Sample**" – "**Unit**" (mg/µl/mio cells/number) – "**Sample Name**" (your individual sample ID/name).

- Remaining samples are **discarded two weeks** after data has been sent to the user without additional notice.
    - Please inform us beforehand if you intend to collect remaining sample material. **The user is responsible to pick-up remaining samples within this time frame.**
    - To arrange pick-up, please contact **metabolomics-samples@uni-heidelberg.de**

- After the results have been send to the user, the data will be **stored** by MCTP in copy for **two months**. The user is responsible for long-term data storage.

Specifications for the analyses of liquid samples

- Include a "pure" medium sample that can serve as "blank" or for spiking experiments.
- Please provide the optimal amount that is recommended, do not overfill vials and tubes.
- 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, metals) as this might significantly alter retention time, baseline or signal strength (matrix effects) or even contaminate the MS systems.
- **Filtrate all liquid samples prior submission using 0.2µm filters.**
    - If this is not possible please contact us.

Specifications for the analyses of solid samples

- Please deliver all samples **without any supernatant** as this significantly alters results
- Solid **samples must be pulverized**
    - E.g. by mortar or ball mill using liquid nitrogen.
      Take care that the samples remain deep-frozen at all times during grinding!
    - We do not accept whole, non-processed solid samples
    - **Record the sample weight or cell numbers for normalization**

- Samples must be sent or handed over in **plastic or paper boxes**
    - **Not accepted** are plastic bags, falcon tubes or lose in a dry ice box
    - Exceptions are made for culture plates.

Recommendations for sample numbers (biological replicates) per sample group

	Cell Culture	Small Animals/ Plants	Human Studies
Optimal	**>6**	**>10**	**>50**
Rigorous	**5-6**	**8-10**	**40-50**
Acceptable	**3-4**	**5-7**	**25-40**

Method development for targeted analyses

Method development is highly dependent on the scientific project. Therefore, a project discussion is mandatory to set expectations, time frame and discuss limitations before starting any method development.

Due to the case-by-case nature, development needs to be charged according to expenditure of time and chemicals/materials/test samples used that is estimated before project start.

Determination of recovery rates 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.

For absolute metabolite quantifications (besides established methods), we kindly request the following:

1. Provide an aliquot of the pure analytes.
    - Upon agreement with the customer, chemicals can also be purchased by the MCTP at the expense of the user.
2. Provide their molecular weight.
3. If available, provide the approximate concentration of the metabolites of interest in your sample. This will significantly advance method development and preparation of standards.
4. Provide us with any additional information about the detection/separation of the metabolites of interests that are known to you.

Method validation

Validation of analytical methods is in general only important for pharmaceutical analysis when insurance of the continuing efficacy 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 usually NOT necessary to use fully validated methods.

Sample transfer

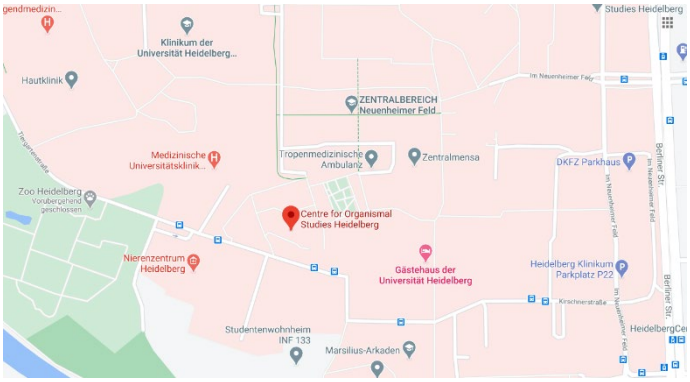
- Sample tubes must be sent or handed over in **plastic or paper boxes.**
    - **Not accepted** are plastic bags, falcon tubes or lose in a dry ice box.

- Submit your samples during our official sample submission times as listed in the online booking system. For submission outside of these times please contact: metabolomics-samples@uni-heidelberg.de

  Avoid sending samples via post on Thursdays or Fridays.

- Samples must remain deep-frozen during transport, e.g. dry ice or liquid nitrogen.

Address for shipping:
Metabolomics Core Technology Platform
COS – University of Heidelberg
Im Neuenheimer Feld 360
69120 Heidelberg
Germany



Disclaimer

While we reserve the right to refuse processing samples if they **do not adhere** to the requirements stated in this document. In case of uncertainties or questions, please do not hesitate to reach out to us prior to sample preparation or submission.

Sample quantities and vessel by sample type

Cell pellets

specific Part/Tissue	Analysis	Optimal amount (minimal amount) *10^6	Container
E.g. Blood cells, Murine cells, Human cell lines, Cancer cells	Biocrates MxP Quant 500	**5** (3)	
	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)
	Nucleotides	**5** (3)	
	TCA cycle compounds	**3** (1)	
	Thiols	**3** (1)	
	Tryptophan and Trp-related metabolites	**5** (1)	
	GC-MS Profiling	**6** (4)	
Primary cells	Biocrates MxP Quant 500	**8** (5)	
	Adenosines	**2** (1)	
	AA + Polyamines	**2** (1)	
	α-Ketoacids	**3** (2)	
	Nucleotides	**5**	
	TCA cycle compounds	**3** (2)	
	Thiols	**3** (2)	
	Tryptophan and Trp-related metabolites	**6** (3)	
	GC-MS Profiling	**10** (8)	

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 / Biofluids

LIQUID SAMPLES	Analysis	Optimal amount (minimal amount) µl	Container
E.g. cell culture supernatants, bacterial supernatants, serum/plasma, etc.	Biocrates MxP Quant 500	**50** (30) Maximum: 200	1.5ml Tube "Safe Seal"
	Adenosines	**250** (100)	
	AA + Polyamines	**100** (25)	
	Anions	**70** (35)	1.5ml Screw top tube (heat-resistant)
	α-Ketoacids	**200** (60)	1.5ml Tube "Safe Seal"
	Nucleotides	**100** (25)	1.5ml Tube "Safe Seal"
	TCA cycle compounds	**150** (30)	
	Thiols	**150** (30)	
	Tryptophan and Trp-related metabolites	**100** (50)	
	Urea	**350** (150)	
	Total fatty acids (TFA)	**100** (50)	
	GC-MS Profiling	**200** (100)	

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

Tissue samples

Tissue specific Part/Tissue	Analysis	Optimal amount (minimal amount) mg	Container
E.g. liver, kidney, heart, spleen, brain, lung, muscle, tumor tissue	Biocrates MxP Quant 500	**30** (10) Maximum: 50	
	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)	
	GC-MS Profiling-	**50** (20)	

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.

Plant samples

Plants specific Part/Tissue	Analysis	Optimal amount (minimal amount) mg	Container
E.g. seeds, seedlings, leaves, stems, roots	Biocrates MxP Quant 500	**30** (20)	
	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)	
	GC-MS Profiling	**50** (30)	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.