Sample preparation guidelines

for Untargeted Metabolomics

Ver. 1.0

University of Utah – Metabolomics Core

Adapted from Lipotype, Inc.

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**Your experiment is precious and unique. The following guidelines are only general guidelines for sample collection. The proper preparation of samples is crucial to obtaining high-quality data and minimizing turnaround time. Therefore, please contact us before you collect or aliquot samples –** Leon.Catrow@utah.edu

**\*\*\*FOR BEST RESULTS, PLEASE CONSULT WITH THE METABOLOMICS CORE PRIOR TO BEGINNING ANY EXPERIMENTAL DESIGN OR SAMPLE COLLECTION.\*\*\***

# List of samples

For sample management, we need a sample list in a specified format (Table 1). You will receive an excel template that will help with preparation.

Please remember:

* **Send your list of samples as an email attachment before you send the samples.**
* Sample “short name” is the identifier we use while handling samples. Ensure that tube labels exactly match the “shortName” in the sample list.
* For sets of samples bigger than 50 samples, we advise that you randomize samples within a set. That is, the order of samples should be random and should not reflect a sequence of conditions, cohorts etc. Please consult us on this topic before you arrange your set.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **ShortName TubeID** | **FullName** | **Cohort** | **Description** | **BiologicalOrigin** | **Amount (uL, mg or cell count)** |
| A1 | Cells sample 1 | KO | cells collected in 2023 with treatment X | H. sapiens | 100 |
| A2 | Cells sample 2 | KO | cells collected in 2023 with treatment X | H. sapiens | 100 |
| B1 | Cells sample 3 | control | cells collected in 2023 with treatment X | H. sapiens | 100 |
| B2 | Cells sample 4 | control | cells collected in 2023 with treatment X | H. sapiens | 100 |
|   |   |   |   |   |   |
|   |   |   |   |   |   |

Table 1. Sample list example. You will receive a template with instructions to prepare your own.

**-Short Name Tube ID:** Maximum 4 characters. Must be unique. Appears on tube labels, plots, and data tables.Label both tube bodies and caps with permanent marker. **DO NOT USE STICKERS OR TAPE LABELS.** Ensure that your labeling will resist freezing, thawing, condensation of water, storage in the cold, shipment and any other handling.

**-Full Name:** Unique, full name of the sample. Can be the same as the short name if no additional information is needed.

**-Cohort:** Group or condition that samples belong to. Samples from the same cohort are considered replicates. Cohort names are used in plots and data tables. Multiple cohorts can be added if necessary.

**-Description:** Detailed description of the sample, such as a reference to a laboratory protocol or other relevant information.

**-Biological Origin:** Biological origin of the sample (e.g., *M. musculus*, *H. sapiens*, liposomes).

**-Amount:** Delivered volume, mass, or count of the sample in µL, mg, or number of cells.

**-Sample Processing Date:** If samples were processed in separate batches we may use this info to batch correct

**-Notes:** Users can add columns or other miscellaneous information as needed.

**Special Characters:** Do not use special characters like commas, periods, slashes, backslashes, brackets, or underscores. Only dashes (-) are allowed.

**Notes on Containers / Vials**

Using proper vials ensures smooth and safe processing of your samples.

# Sample handling and storage

Samples should be handled according to common laboratory practice ensuring their purity and preservation of their metabolome composition. Please follow all safety practices associated with your samples.

Namely but not exclusively:

* Samples should not be exposed to temperatures above 4 **°**C unnecessarily; long exposures to such temperatures should be avoided.
* Microbiologically and chemically clean labware should be used.
* Samples should be protected from contamination by working in clean, dust-free conditions and always handled with gloves.
* Collected samples should be stored at -80 **°**C (or in liquid nitrogen).
* Unnecessary freezing and thawing should be avoided.

# Containers

Using proper containers ensures smooth and safe processing of your samples. As our procedures are automated we are not as flexible in handling samples as with manual operations. Therefore, please remember that it is very important to deliver samples in recommended containers.

Namely:

* **Please send samples in Eppendorf Safe-Lock Tubes, 1.5 or 2.0 mL, Eppendorf Quality™, colorless (cat# 0030120086 or cat# 0030120094).** [https://www.eppendorf.com/de-de/eShop-Produkte/Spitzen-](https://www.eppendorf.com/de-de/eShop-Produkte/Spitzen-Reaktionsgef%C3%A4%C3%9Fe-und-Platten/Reaktionsgef%C3%A4%C3%9Fe/Eppendorf-Safe-Lock-Tubes-p-PF-8863?gclid=EAIaIQobChMIup_Fl8717gIVXoBQBh1aKAwLEAAYASAAEgLENfD_BwE)

[Reaktionsgef%C3%A4%C3%9Fe-und-](https://www.eppendorf.com/de-de/eShop-Produkte/Spitzen-Reaktionsgef%C3%A4%C3%9Fe-und-Platten/Reaktionsgef%C3%A4%C3%9Fe/Eppendorf-Safe-Lock-Tubes-p-PF-8863?gclid=EAIaIQobChMIup_Fl8717gIVXoBQBh1aKAwLEAAYASAAEgLENfD_BwE)

[Platten/Reaktionsgef%C3%A4%C3%9Fe/Eppendorf-Safe-Lock-Tubes-p-PF8863?gclid=EAIaIQobChMIup\_Fl8717gIVXoBQBh1aKAwLEAAYASAAEgLENfD\_B wE](https://www.eppendorf.com/de-de/eShop-Produkte/Spitzen-Reaktionsgef%C3%A4%C3%9Fe-und-Platten/Reaktionsgef%C3%A4%C3%9Fe/Eppendorf-Safe-Lock-Tubes-p-PF-8863?gclid=EAIaIQobChMIup_Fl8717gIVXoBQBh1aKAwLEAAYASAAEgLENfD_BwE)

* **Please send accurately pre-weighed tissue samples in Qiagen PowerBead Tubes, Ceramic 1.4 mm (50) Cat. No. / ID:   13113-50.** https://www.qiagen.com/us/products/discovery-and-translational-research/lab-essentials/plastics/powerbead-tubes
* If you intend to use different containers or have a larger sample set, please discuss these issues with us before you collect or aliquot samples, if possible.



Figure 2. Recommended tubes: 1.5 mL or 2 mL Eppendorf Safe-Lock tube (cat# 0030120086 or cat# 0030120094).

# Sample labeling

As we handle many samples at the same time it is critical that they are identified easily and unambiguously.

Accordingly:

* Please label tubes in a clear, legible and unambiguous way using a **shortName** corresponding to the sample list.
* Label both lids and tube bodies.
* If you use stickers/scotch tape, ensure that they will not increase the tube diameter significantly. More than one layer around a tube will be too much.
* Ensure that your labeling will resist freezing, thawing, condensation of water, storage in the cold, shipment and any other handling.

# Sample shipment

The shipment of samples is a critical step, as it is during this step that they are technically beyond your and our control.

Please contact us about any details you need to organize the shipment and remember:

* **You are fully responsible for all duties regarding shipment, including but not limited to: custom and clearance, custom fees, other miscellaneous shipment related fees, and costs.** If your samples are of animal origin or sent in a serum

of animal origin, they will require an import permit.

* **Do not forget to correctly describe/declare samples, such that the carrier (or any other party involved) does not need to contact us and to avoid problems at customs.**
* Samples should be properly sealed and packaged. The sample tubes should be placed in cardboard cryoboxes, rather than bags. **DO NOT SHIP TUBES LOOSE IN DRY ICE.**
* Samples should be shipped frozen and it should be ensured that they arrive frozen. We recommend using a bigger box with more dry ice to ensure continuous cooling for the entire duration of the shipment process.
* Please arrange with us beforehand for when to send your samples and when we should expect them.

Shipping address:

**University of Utah**

**Metabolomics Core**

**15 N Medical Drive East, RM A306**

**Salt Lake City, UT 84112-5650 USA**

# Preparing samples

## General remarks

Below are presented suggested protocols for preparing certain types of biological samples, which based on our experience, are known to be compatible with our procedures and techniques. However, whatever kind of material you intend to have analyzed by us, some general rules need to be followed.

Namely:

* All chemicals and solvents used should be of LC, LC/MS or higher quality grade.
* **Samples have to be completely homogenous, i.e. no clots, clumps etc.**
* Minimum volume to be delivered cannot be lower than indicated, but usually it can be higher. **If the minimum volume cannot be achieved, please contact the core prior to submitting samples.**
* All samples should be the same or very similar in their concentrations, cell numbers, or OD units.
* Strictly follow recommended sample concentrations.

**\*\*\* CONSULT WITH THE METABOLOMICS CORE PRIOR TO BEGINNING ANY EXPERIMENTAL DESIGN OR SAMPLE COLLECTION.\*\*\***

## Blood plasma/serum and erythrocyte samples

To obtain **fresh** **plasma** and **erythrocyte** samples, the following protocol should be used:

1. Transfer **300 µL** of fresh blood stabilized with an anticoagulant to 2 mL Eppendorf tube.
2. Spin down; 2000 g, 10 min.
3. Take **75-150 µL** of the supernatant without disturbing red blood cells pellet and transfer it to a fresh 2 mL Eppendorf tube. **This is Plasma sample.**
4. Discard the remaining supernatant. Try not to collect too much of red blood cells, but still aspirate a very small volume from the top of the pellet layer to remove the whitish buffycoat (the leukocyte interlayer).
5. **Fill tubes up to 1.5 mL** with "D-PBS without Mg, Ca"; resuspend well.
6. Transfer **300 µL** to fresh 2mL Eppendorf tube.
7. Add **1.2 mL** of "D-PBS without Mg, Ca"; resuspend well.
8. Spin down; 2000 g, 10 min; discard the supernatant.
9. Add **1.2 mL** of "D-PBS without Mg, Ca"; resuspend well.
10. Spin down; 2000 g, 10 min; discard the supernatant.
11. Retain the packed erythrocytes. **This is Erythrocytes sample.**
12. Freeze all samples at -80 °C.

Notes:

* D-PBS without Mg, Ca: Dulbecco's phosphate-buffered saline without magnesium and calcium.
* All samples have to be completely homogenous, i.e. no clots, cell clumps etc.
* It is acceptable for plasma (or serum) and erythrocyte samples to have the final volume between 25 µL and 1 mL, as long as the raw material concentrations dictated by the above instruction are preserved.
* Additional information about general handling of blood and blood-derived samples can be found in:

Jimmie B Vaught, “Blood Collection, Shipment, Processing, and Storage.” *Cancer Epidemiology, Biomarkers & Prevention* 15, no. 9: 1582–4, 2012. doi:10.1158/1055-9965.EPI-06-0630.

## Yeast and bacteria

To obtain yeast or bacteria samples for total cell metabolomics, the following procedure should be used.

1. Spin down yeast or bacteria cells; 5000 g, 3 min. **Record the OD.**
2. Wash cells twice in water and spin down; 5000 g, 3 min.
3. After the last wash, the pellet can be snap-frozen in liquid nitrogen and stored at -80 °C or processed immediately. **Submit the pellet to the Metabolomics Core, which will handle any additional extraction.**

Notes:

* All samples have to be completely homogenous, i.e. no cell clumps etc.

## Cultured and isolated cells

To obtain cell samples for total cell metabolomics, the following procedure should be used.

1. Collect cells using a method appropriate for a given cell type.
2. Wash collected cells twice by resuspending them in “D-PBS without Mg, Ca” and spinning down using speeds appropriate for a given cell type.
3. After the last wash, the pellet can be snap-frozen in liquid nitrogen and stored at -80 °C or processed immediately. **Submit the pellet to the Metabolomics Core, which will handle any additional extraction.**

Notes:

* D-PBS without Mg, Ca: Dulbecco's phosphate-buffered saline without magnesium and calcium
* All samples have to be completely homogenous, i.e. no cell clumps etc.
* Cell pellets should contain between 5 x 103 and 1 x 107 cells, depending on the desired metabolites to be observed.
* To ensure comparability across samples, normalization is essential. Please normalize your samples using **accurate cell counts** or **total protein or DNA content**. We strongly prefer accurate cell counts. If you choose to normalize by protein, we prefer that you quantitate it yourself prior to submission; however, we can perform protein quantitation in the lab for a nominal charge if needed.

## Tissues and organs

To prepare tissue samples for untargeted metabolomics, the following procedure should be used.

1. Pre-weigh a Qiagen Powerbead tube prior to sample collection.
2. Collect 20-40 mg of homogenous tissue, snap freeze, and transfer to the pre-weighed Qiagen Powerbead tube.
3. Record the weight of tissue collected.
4. Store at -80 °C prior to submission.

Notes:

* All samples have to be completely homogenous, i.e. no clots, clumps, pieces of a connective tissue.
* All organ and tissue samples should be collected from the same area of the organ.
* It may be necessary to combine small organs to meet the minimum required tissue amount.

## Purified organelles, sub-cellular preparations and similar material

To obtain such sample types please, the following procedure should be used.

1. Follow your protocols to obtain a given material.
2. Store a snap frozen pellet at -80 °C prior to submission.

Notes:

* All samples have to be completely homogenous, i.e. no clumps etc.
* Ensure that protein concentration measurements are compatible with the sample type and buffers used.
* Ensure that the protein concentration is measured for a properly diluted sample (in the method’s dynamic range).
* Be advised that some buffers used for sub-cellular preparations are not compatible with metabolomics analysis, including but not limited to: high salt and high detergent buffers, buffers containing polymers and buffers with lipophilic substances.
* If water is not suitable for your preparations use D-PBS without Mg, Ca (Dulbecco's phosphate-buffered saline without magnesium and calcium).

## *Drosophila melanogaster* - fruit fly and its organs/tissues

To obtain *Drosophila* samples, the following procedures should be used. The amounts below are a general guideline; however, please contact the metabolomics core to ensure that the amounts are appropriate for the desired analysis.

1. Collect the following amounts of material for each sample:

* For a **whole organism** metabolome:

 **Stage Qty**

|  |  |
| --- | --- |
| Larvae  | 100-1000 larvae  |
| Pupae  | 10-40 pupae  |
| Adult  | 10-40 adult flies  |

* For a metabolome of an organ/tissue **originating from the 3rd instar larvae or an adult fly**:

|  |  |
| --- | --- |
| **Tissue**  | **Qty**  |
| Gut  | 20-40 pcs  |
| Fat Body  | 10 pcs  |
| Salivary glands  | 6 pairs  |
| Wing disk  | 60 pcs  |
| Brain  | 20-40 pcs  |

####  Hemolymph 10-20 µL

1. Transfer flies or organics into a pre-weighed Qiagen Powerbead tube and snap-freeze. It is useful to record sample weights for possible future use in normalization.

Notes:

* All samples have to be completely homogenous, i.e. no cell clumps etc.
* Different quantities might be required for organs/tissues originating from other developmental stages.

## Cerebrospinal fluid (CSF)

To obtain CSF samples, the following procedure should be used.

 • Collect at least **200 µL** of CSF in Eppendorf tube and freeze it at -80 °C.

## Samples of other origin

To submit samples not covered here, please consult the Metabolomics Core.