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Standard Operating Procedures (SOP) for metabolomics

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Content

Harvesting and collection of adherent cells

General considerations

Adherent cells should be grown in cell culture plates (e.g. 6-well) and not in flasks.

The suitable cell number depends on the cell line and aim of the study. It should be evaluated prior to the according experiment. A **cell number of 2.5 x 10^6** is a good starting point. It might be necessary to pool wells to reach appropriate cell numbers as shown in Figure 1.

The required volume for analysis of cell lysate is at least **1 mL of lysate per sample**. It might be necessary to pool wells to reach an appropriate volume as shown in Figure 1.

For analysis of supernatant medium a volume of at least **0.5 mL of cell-free supernatant per sample** is required.

A **method for data normalization** to actual cell numbers and growth rate should be considered and performed prior to metabolomics analysis. Different methods may be applied, such as, but not limited to, protein concentration or Hoechst assay. Figure 1 shows an example of how to harvest cells for normalization.

Please provide a filled out **request for analysis** form and a filled out **check list** when sending samples to the MS-facility.

Label every single tube with project name, sample number, etc. Make sure that labelling is waterproof and resistant to cold storage conditions. Store the vials in labelled boxes (no bags), ordered by sample number (corresponding to your sample list).

Please keep your processing procedures and times standardised and use the same kind of collection and storage tubes for all samples of one study to assure comparability of results. Avoid thawing of frozen samples: always use dry ice during handling and transportation.

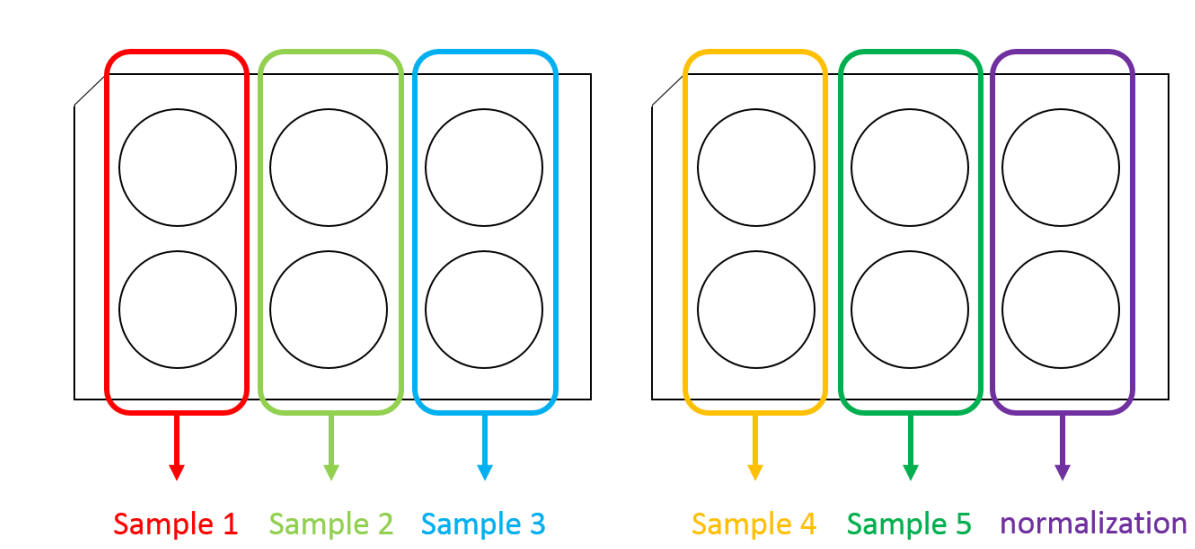

Figure 1: Example for pooling wells from different plates (but same group) to achieve appropriate cell numbers and volume.

Sample collection of medium (cell culture supernatant)

Skip this step if analysis of medium is not required.

All volumes given below refer to a 6-well plate. When using other plates volumes should be scaled appropriately.

- Harvest medium well by well.
- Collect 1 mL of medium per sample. If necessary combine aliquots from different wells.
- Centrifuge medium at 1000 x g and room temperature for 10 min.
- Carefully transfer 0.5 mL of the cell-free supernatant into a fresh Eppendorf tube and immediately place the tube on dry ice or snap-freeze in liquid nitrogen.

For control purpose please provide blank medium which was incubated the same way but without cells.

After harvesting immediately store samples at -80°C. Please send samples on dry ice and in one batch to the MS-facility.

Sample collection of cells (cell lysate)

Extraction solvent (80% v/v methanol) is provided by MS-facility and contains four recovery standard compounds. The solvent should be stored at -80°C.

All volumes given below refer to a single well of a 6-well plate. When using other plates volumes should be scaled appropriately. 1 mL of cell lysate is required per sample, so pooling of lysate from different wells may be necessary for one sample. Please see Figure 1 for an example.

- Harvest the cells well by well.
- Remove the (remaining) medium.
- Wash the cells two time with PBS (37°C). Use at least 1 mL more PBS than culture medium to ensure proper washing of the well (e.g., if the growth medium volume for the 6-well is 2 mL, then use 3 mL PBS for washing). Remove the PBS after the second washing step as completely as possible.
- Add 350 µL of precooled ice cold (dry ice) extraction solvent to the 6-well and ensure that all cells are covered immediately. The extraction solvent can be difficult to pipette when cold as it has a viscous consistency at low temperatures.
- Detach the cells using a rubber-tipped cell scraper and transfer the cell suspension into precooled (dry ice) Eppendorf tube. The tubes should be precooled in dry ice and handled on dry ice during the whole procedure.
- Add another 350 µL of ice cold (dry ice) extraction solvent to wash the well and transfer the wash volume to the tube as well. Immediately place the tube on dry ice or snap-freeze in liquid nitrogen.

After harvesting immediately store samples at -80°C. Please send samples on dry ice and in one batch to the MS-facility.

Harvesting and collection of suspension cells

General considerations

Suspension cells should be grown in cell culture flasks.

The suitable cell number depends on the cell line and aim of the study. It should be evaluated prior to the according experiment. A **cell number of 2.5 x 10^6** is a good starting point.

Cells for analysis are required as **cell pellets**.

For analysis of supernatant medium a volume of **at least 0.5 mL of cell-free supernatant per sample** is required.

A **method for data normalization** to actual cell numbers and growth rate should be considered and performed prior to metabolomics analysis. Despite counting suspension cells prior to harvest, certain cell losses occur during extensive washing steps. Different methods may be applied, such as, but not limited to, protein concentration or Hoechst assay.

Please provide a filled out **request for analysis** form and a filled out **check list** when sending samples to the MS-facility.

Label every single tube with project name, sample number, etc. Make sure that labelling is waterproof and resistant to cold storage conditions. Store the vials in labelled boxes (no bags), ordered by sample number (corresponding to your sample list).

Please keep your processing procedures and times standardised and use the same kind of collection and storage tubes for all samples of one study to assure comparability of results. Avoid thawing of frozen samples: always use dry ice during handling and transportation.

Sample collection of medium (cell culture supernatant)

Skip this step if analysis of medium is not required.

- Gently mix the cell suspension.
- Collect 1 mL of cell suspension per sample in an Eppendorf tube. If necessary combine aliquots from different flasks.
- Centrifuge cell suspension at 500-800 x g and room temperature for 5-10 min. Centrifugal force and centrifugation time depend on cell line and needs to be adapted so that the cell pellet is firmly separated from the supernatant. At higher centrifugation forces cells may be destroyed and their content may leak into the medium.
- Carefully transfer 0.5 mL of the cell-free supernatant into a fresh Eppendorf tube and immediately place the tube on dry ice or snap-freeze in liquid nitrogen.

For control purpose please provide blank medium which was incubated the same way but without cells.

After harvesting immediately store samples at -80°C. Please send samples on dry ice and in one batch to the MS-facility.

Sample collection of cells (cell pellet)

- Gently mix the cell suspension.
- Collect an appropriate volume of cell suspension and count the cells.
- Transfer a volume containing the required cell number to an Eppendorf tube. If necessary combine aliquots from different flasks.
- Centrifuge cell suspension at 500-800 x g and room temperature for 5-10 min. Centrifugal force and centrifugation time depend on cell line and need to be adapted so that the cell pellet is firmly separated from the supernatant. At higher centrifugation forces cells may be destroyed and their content may leak into the medium. Remove the supernatant as completely as possible and gently re-suspend the pellet in warm PBS (37°C). Use at least 1 mL more PBS than culture medium to ensure proper washing. Perform two washing steps. After the second washing step remove the PBS as completely as possible and immediately place the tube on dry ice or snap-freeze in liquid nitrogen.

After harvesting immediately store samples at -80°C. Please send samples on dry ice and in one batch to the MS-facility.

Collection of plasma, serum, and urine samples

General considerations

Please provide a filled out **request for analysis** form and a filled out **check list** when sending samples to the MS-facility.

Label every single tube with project name, sample number, etc. Make sure that labelling is waterproof and resistant to cold storage conditions. Store the vials in labelled boxes (no bags!), ordered by sample number (corresponding to your sample list).

Please keep your processing procedures and times standardised and use same kind of collection and storage tubes for all the samples of one study to assure comparability of results. Avoid thawing of frozen samples: always use dry ice during handling and transportation.

Collection of plasma samples

The preferred anticoagulant for plasma preparation is EDTA, but also heparin is acceptable. It is not recommended to use citrate.

- Collect blood samples from a peripheral vein directly into tubes. Shake the tubes – gently, but thoroughly – after finishing blood collection. Do not cool blood before plasma separation is finished.

- Separate cells and plasma using centrifugation as soon as possible. Time from blood collection to centrifugation must not exceed 40 min. Spinning-conditions are as follows: 20-24 °C, 10 min at 4500 x g (mouse blood) or 2750 x g (human blood).

- Label sample storage tubes (for labelling see general considerations). Cool sample storage tubes and perform pipetting steps on ice.

- Transfer the plasma into a pre-cooled collection tube without aspirating blood cells; use disposable pipette tips. Vortex plasma and place on ice. Aliquot the plasma in suitable portions into the labelled sample storage tubes to avoid later freeze/thaw cycles. The filling of the vials must not exceed three-quarters of their capacity. The minimal filling is depending on the tubes used.

- Plasma samples need to be frozen immediately (e.g. in liquid nitrogen) and stored at -80°C. Store the tubes in boxes (no bags), ordered by sample number.

Collection of serum samples

- Collect blood samples from a peripheral vein into the serum collection tube with clotting activator.

- After finishing blood collection, shake the tube – gently, but thoroughly.

- Store the tube at room temperature (20-28 °C) in an upright position to allow coagulation. Clot formation should be completed after 20-30 min in most cases. If centrifugation is not performed at the place of sample collection, use this time for transportation. Time at room temperature until centrifugation should not exceed 40 min.

- Centrifuge to separate the serum from the blood clot (15 °C, 10 min, 4500 x g (mouse blood) or 2750 x g (human blood)).

- Label sample storage tubes (for labeling see general considerations). Cool sample storage tubes and perform pipetting steps on ice.

- Transfer the serum into a pre-cooled collection tube without aspirating blood cells. Use disposable pipette tips. Vortex serum and place on ice. Aliquot the serum in suitable portions into the pre-cooled, labeled storage tubes to avoid later freeze/thaw cycles. The filling should not exceed three-quarter of tubes capacity. The minimal volume is depending on the tubes used.

- Serum samples need to be frozen immediately (e.g. in liquid nitrogen) and stored at -80°C. Store the tubes in boxes (no bags), ordered by sample number.

Collection of urine samples

- Collect urine and note the total volume as exactly as possible.
- After finishing urine collection, shake the collection vessel – gently, but thoroughly.
- Centrifuge aliquots of the urine (15 °C, 10 min, 4500 x g).
- Label sample storage tubes (for labeling see general considerations). Cool sample storage tubes and perform pipetting steps on ice.
- Transfer aliquots of the supernatant into a pre-cooled collection tube. Use disposable pipette tips. Vortex urine and place on ice. Aliquot the urine in suitable portions into the pre-cooled, labeled storage tubes to avoid later freeze/thaw cycles. The filling should not exceed three-quarter of tubes capacity. The minimal volume is depending on the tubes used.
- Urine samples need to be frozen immediately (e.g. in liquid nitrogen) and stored at -80°C. Store the tubes in boxes (no bags), ordered by sample number.

Version history

Version	Date	Changes
1.0	06. Aug. 2019	New document
2.0	08. Jan. 2020	Updated version (extraction solvent volume changed)