# 代谢组学样本准备指南

## *1 Preparation of before sample collection*

1) Check of possible contaminants from the collectors and tubes

Collector: add 1 mL artificial serum to sample collectors and leave at room temperature for 1 hour. Then add 3 mL ACN to artificial serum. After vortexing for 30 seconds, centrifuge the mixture at 14000×g for 10 min at 4 ℃. Pipe the supernatant for analysis.

Tube: add 200 μL 80% methanol (v/v) to the tubes and leave at room temperature for 4 hours. Centrifuge the mixture at 14000×g for 10 min at 4 ℃. Pipe the supernatant for analysis.

Pipette tip: pipe 80% methanol (v/v) with different pipette tips at room temperature for 50 times. Centrifuge the mixture at 14000×g for 10 min at 4 ℃. Pipe the supernatant for analysis.

2) Preparation of donor/participants

A 12 hours of fasting is required for all participants. No unrelated drugs or alcohol intake within this 12 hours. Keep quiet, no unnecessary physical exercise before sample collection,.

## *2 Collection of plasma*

1) Collect whole blood in tubes containing EDTA as anti-coagulant.

2) Mix the blood after collection, prior to centrifugation. After mixed, centrifuge the samples at 1,000 g for 10-15 minutes. Avoid hemolysis.

3) Transfer the clear, upper layer of plasma to a new clean Eppendorf tube. The volume of plasma per tube should be 100 to 200 μL.

### *3 Collection of serum and other biofluids*

1) Collection of serum

Collect whole blood in serum separator tubes and follow tube manufacturer's processing instructions.

Transfer the straw-colored or pale-yellow upper layer of serum to a new clean Eppendorf tube.

2) Collection of biofluids (urine, saliva, cerebrospinal fluids, etc)

Collect biofluids according to routine clinical protocols.

Centrifuge the biofluids at 1000×g, 5 minutes at 4 ℃.

Transfer the supernatant into clean Eppendorf tube.

### *4 Collection of tissue*

1) Cut target tissues (20 to 50 mg) directly or within 30 minutes after clinical resection.

2) Flush the samples with PBS (4℃) to remove blood or other contaminants.

3) Transfer the samples to cryovial and flash-freeze in liquid nitrogen.

### *5 Collection and preparation of cell culture*

1) Change the culture medium 2 hours before cell collection

2) For adherent cell lines, remove the medium rapidly, and rinse the cell gently with 5.0 mL PBS twice (37 ℃).

3) For suspension cell lines, remove the medium with centrifuge at 125×g, 4 minutes. Then rinse the cell gently with 5.0 mL PBS (37 ℃) and remove the PBS with centrifuge at 125×g, 4 minutes twice.

4) Add 2 mL 80% methanol with IS (V/V, cool to – 80 ℃) to the cell.

5) Incubate the mixture at – 80 ℃ for 5 minutes.

6) Scrape the cell (adherent) from the dish on dry ice.

7) Transfer the mixture to 15 mL conical tube on dry ice.

8) Lyse the cell with two cycle of freeze-thaw (frozen in liquid nitrogen and thaw at 37 ℃).

9) Vortex the mixture for 1 minute.

10) Centrifuge the mixture at 14000 ×g, 10 minutes , at 4 ℃.

11) Remove the supernatant to a new tube.

12) Add 500 μL 80% methanol to the cell pellet and repeat step 3, 4 and transfer the supernatant to the same tube.

13) Lyophilize the extraction solvent and stored at – 80 ℃ until analysis.

## *6 Storage and transportation of samples*

1) Biofluid samples should be stored in – 80 ℃ immediately after collection. Tissues and cell are recommended to be stored in liquid nitrogen. All samples in same project should be collected and stored at the same conditions.

2) All samples are recommended to be divided into aliquots for a single analysis. Avoid frequent freeze-thaw cycles (less than 3 times at 4 ℃ is recommended).

3) During the transportation, dry ice should be used to preserve the biological samples.