Gereal
Biotechnology Of Life Sciences

MULTI-OMICS CENTER

Sample Collection & Handling Guide

Metabolomics · Metabolic flux tracing · Proteomics · Sequencing · Single-cell · Molecular & cell assays

> This guide lists recommended amounts, collection and processing steps, key parameters and common mistakes by service type for pre-submission self-checks. Where it differs from the Sample Collection Guide confirmed for your project, the project protocol prevails.

1. Metabolomics (untargeted / targeted / lipidomics) LC-MS

Sample type	Amount	Collection & handling
Serum / plasma	≥ 100 µL	Fasting draw, EDTA or heparin; centrifuge 3000 g 10 min at 4 °C, aliquot and freeze within 2 h
Tissue	≥ 50 mg	Rinse off blood, snap-freeze in liquid nitrogen; avoid freeze–thaw cycles
Cells	≥ 1×10^6	Wash twice with PBS to remove medium, aspirate and snap-freeze
Urine	≥ 200 µL	Mid-stream, spin down debris, aliquot and freeze
Faecal	≥ 100 mg	Collect in sterile tube, −80 °C within 2 h, avoid temperature cycling
Plant / microbial	≥ 100 mg	Grind in liquid nitrogen and freeze; note culture conditions for microbes

Collection notes

- Keep sample type and anticoagulant consistent within a project; never mix serum and plasma
- Diet affects metabolites — standardise fasting time for clinical samples and record it
- Avoid buffers with detergents or high salt, which interfere with chromatography
- Aliquot into single-use volumes to eliminate repeat freeze–thaw

Note: targeted assays size the sample by the analyte list; low-abundance compounds may need larger volumes — confirm at design stage.

Step-by-step: serum / plasma

1. Draw after 8–12 h fasting; keep the anticoagulant consistent (EDTA or heparin) — use a clot activator tube for serum
2. Serum: stand at room temperature for 30 min. Plasma: mix gently 5–8 times immediately
3. Centrifuge at 3000 g for 10 min at 4 °C and transfer the supernatant, avoiding the buffy coat and any haemolysis

4. Aliquot 100–200 µL per tube, label with ID and time, move to −80 °C within 2 h
5. Ship on dry ice, keeping below −70 °C; dry ice must not run out before arrival

Step-by-step: tissue and cells

1. Rinse tissue in pre-chilled PBS within 5 min of collection; remove fat and connective tissue
2. Cut into pieces of ≤ 50 mg, snap-freeze in liquid nitrogen for at least 30 s, then store at −80 °C
3. Wash cells twice with pre-chilled PBS to remove medium (residual serum interferes with chromatography)
4. Aspirate the supernatant and snap-freeze the pellet — do not freeze cells in PBS

Common mistakes

- × Mixing serum and plasma or changing anticoagulants → groups are not comparable
- × Repeated freeze–thaw of one aliquot → metabolite degradation and poor reproducibility
- × High-salt or detergent-containing buffers → ion suppression and column contamination
- × Using haemolysed samples for targeted assays → falsely elevated intracellular analytes

2. Metabolic flux & isotope tracing 13C / 15N

Sample type	Amount	Collection & handling
Cells (tracer culture)	≥ 2×10^6 per time point	Quench immediately at the endpoint (liquid nitrogen or cold methanol), keep on ice
Tissue	≥ 100 mg	Snap-freeze at the endpoint and record the exact sampling time
Culture medium	≥ 200 µL	Collect at the same time points, filter and freeze for substrate consumption

Collection notes

- Design time points in groups (e.g. 0 / 15 min / 1 h / 4 h) and fix the sampling order in the protocol
- Quenching speed determines whether real flux is captured — process at low temperature immediately
- Always keep an unlabelled control from the same batch for natural-abundance correction
- Record tracer lot, concentration and addition time for reproducibility

Note: tracer, labelling duration and quenching are settled in a pilot run and define which pathways can be resolved.

Labelling workflow

1. Settle tracer concentration, labelling duration and quenching in a pilot run
2. At the target confluence, switch to fresh medium containing the tracer and record T0
3. Sample at each designed time point and quench each independently — never pool first
4. Collect the cell pellet and the medium separately, label and freeze

5. Always keep an unlabelled control group for natural-abundance correction

Key parameters

- Time points: at least 3–4 (e.g. 0 / 15 min / 1 h / 4 h) covering the rising phase, not only steady state
- Replicates: ≥ 3 biological replicates per time point, each started from an independent culture
- Quenching: liquid nitrogen or cold methanol (≥ 4 volumes) at −20 °C, all work on ice
- Records: tracer lot, volume added, culture temperature and exact sampling time

Common mistakes

- × Sampling all time points from one culture → the cells have already changed state
- × Labelling for 24 h → the system nears steady state and flux information is lost
- × Incomplete quenching → enzymatic reactions continue and the MID shifts

3. Proteomics (DIA / PTM / PRM) LC-MS/MS

Sample type	Amount	Collection & handling
Cells	≥ 1×10^6	Wash off serum with PBS, lyse directly or snap-freeze
Tissue	≥ 50 mg	Remove blood and connective tissue, snap-freeze then −80 °C
Serum / plasma	≥ 50 µL	Avoid haemolysis, aliquot and freeze; depletion decided per project
FFPE sections	≥ 4 sections (5 µm)	Note block age and staining history, store dry at room temperature

Collection notes

- Use low-binding tubes and wear gloves/mask to avoid keratin contamination
- Denature soon after lysis to limit protease degradation
- For PTM work keep matched controls and avoid phosphate buffers
- For PRM validation, provide the target protein list to guide method development

Note: protein samples are highly sensitive to freeze–thaw — aliquot so each tube is thawed once.

Sample processing

1. Cells: wash twice with PBS, then lyse directly (with protease inhibitors) or snap-freeze the pellet
2. Tissue: remove blood and connective tissue, weigh, add lysis buffer at 1:5–1:10 (w/v), homogenise on ice
3. Serum/plasma: avoid haemolysis; depletion of abundant proteins is decided per project
4. Centrifuge lysate at 12000–15000 g for 10–15 min at 4 °C and take the supernatant for quantification
5. Aliquot 100–200 µg per tube, store at −80 °C, ship on dry ice

Notes for PTM projects

- Phosphorylation: avoid phosphate buffers throughout; include matched controls

- Acetylation/ubiquitination: add deacetylase/deubiquitinase inhibitors and denature soon after lysis
- FFPE: state block age, fixation time and staining history; 4+ sections at 5 μm
- PRM: provide target protein/peptide lists to guide method development and RT confirmation

Common mistakes

- × No gloves/mask → keratin contamination masks low-abundance proteins
- × Lysates left at room temperature → protein degradation and loss of modifications
- × Estimating input from wet weight with high water/fat content → insufficient protein

4. Sequencing (RNA-seq / amplicon / metagenomics / WGS) NGS

Sample type	Amount	Collection & handling
Total RNA (RNA-seq)	≥ 1 μg, RIN ≥ 7	Snap-freeze, ship on dry ice, avoid RNase contamination
Tissue in RNAlater	≥ 50 mg	Cut into ≤ 5 mm pieces, soak in RNAlater, 4 °C overnight then −20 °C
Faecal / soil (amplicon, metagenomics)	≥ 200 mg	Collect sterile, store at −80 °C, avoid repeated freeze–thaw
Whole blood (WGS)	≥ 500 μL	EDTA only (never heparin); 4 °C short-term, −80 °C long-term

Collection notes

- RNA integrity matters more than concentration — degraded RNA should not be sequenced
- Record sampling site, time and environment for microbial samples
- Use one library protocol within a batch to reduce batch effects
- Ensure dry ice lasts until delivery

Note: amplicon and metagenomic work is sensitive to contamination — use sterile consumables and include blank controls.

RNA workflow

1. Cut tissue into ≤ 5 mm pieces immediately, snap-freeze or immerse in RNAlater (4 °C overnight, then −20 °C)
2. Measure concentration and RIN; RIN ≥ 7 is required for library prep
3. Store RNA at −80 °C, avoid freeze–thaw, ship on dry ice
4. Keep a backup aliquot against shipping loss

Microbial and DNA workflow

1. Faecal/soil: collect ≥ 200 mg in a sterile tube, label, −80 °C within 2 h
2. Include a blank control handled with the same consumables to assess contamination

3. Whole blood: EDTA only — heparin inhibits downstream enzymatic steps
4. DNA: A260/280 within 1.8–2.0, avoid freeze–thaw and prolonged light exposure

Common mistakes

- × Submitting degraded RNA → library failure or 3′ bias
- × Faecal samples left at room temperature → community structure drifts
- × Different library batches within one comparison group → batch effects mask biology

5. Single-cell RNA sequencing scRNA-seq

Sample type	Amount	Collection & handling
Cell suspension	700–1200 cells/μL, viability ≥ 80%	Prepare fresh, transport on ice, load within 4 h
Tissue (to be dissociated)	≥ 100 mg	Store cold immediately after collection; note enzyme tolerance and target cell types
Blood / PBMC	≥ 3 mL whole blood or ≥ 1×10^6 PBMC	EDTA, avoid haemolysis, fresh samples preferred

Collection notes

- Suspension quality drives data quality: no clumps, minimal debris
- Keep dissociation short to avoid stress-response gene expression
- Do not store cells long in Ca^{2+}/Mg^{2+}-containing buffers
- If cryopreservation is required, confirm the protocol and check post-thaw viability

Note: run a small pilot to check viability and cell-type composition before committing to the full run.

Suspension preparation

1. Dissociate at low temperature, briefly and gently; target viability ≥ 80% with no clumps
2. Filter through a 40 μm mesh, centrifuge and resuspend in BSA-containing buffer
3. Adjust to 700–1200 cells/μL and record count and viability
4. Prepare fresh, transport at 2–8 °C and load within 4 h; avoid vigorous agitation

Key parameters

- Target cell number: load 1.5–2× the target capture number
- Keep dissociation ≤ 30 min; longer induces stress genes (Fos/Jun)
- Do not store cells long in Ca^{2+}/Mg^{2+} buffers; never fix samples for live-cell marker staining

Common mistakes

- × Debris- and dead-cell-rich suspension → high empty-droplet rate
- × Loading hours after preparation → viability and transcripts degrade
- × Incomplete dissociation of large tissue pieces → distorted cell-type proportions

6. Molecular & cell assays, pathology (ELISA / WB / flow / sections) Assay

Sample type	Amount	Collection & handling
Serum / plasma (ELISA)	≥ 50 µL	Avoid haemolysis and freeze–thaw; aliquot once
Cell lysate (WB)	≥ 20 µL, ≥ 20 µg protein	Lyse on ice with protease inhibitors, denature promptly
Whole blood / suspension (flow)	≥ 100 µL	EDTA, analyse within 24 h, list target markers
Tissue (sections)	≥ 0.5 cm³	Fix in 4% PFA for 24–48 h; for frozen work embed in OCT and ship on dry ice

Collection notes

- Under- or over-fixation both mask antigens — follow the recommended fixation time
- For IHC with antibody sourcing, confirm species and clone in advance
- Do not fix samples before staining live-cell markers by flow
- State the required section orientation (transverse/longitudinal) on the submission form

Note: for pathology samples the sampling site drives interpretation — provide an H&E reference slide or imaging localisation where possible.

Per-assay workflow

1. ELISA: serum/plasma ≥ 50 µL, no haemolysis, aliquot into single-use volumes
2. WB: lyse on ice with protease inhibitors, quantify by BCA, aliquot ≥ 20 µg and denature
3. Flow: EDTA whole blood or suspension ≥ 100 µL, analyse within 24 h, list markers and isotype controls
4. Sections: fix in 4% PFA for 24–48 h (≤ 1.5 cm thick), dehydrate and embed in paraffin
5. Frozen sections: embed in OCT with the target face down, ship on dry ice, avoid freeze–thaw

Key parameters

- Fixation: too short and antigens are masked; over 72 h and epitopes are blocked
- Section thickness: 4–5 µm (paraffin), 8–10 µm (frozen) — confirm the purpose
- IHC with antibody sourcing: provide species, clone and expected localisation
- State section orientation and sampling site on the form; attach an H&E reference if possible

Common mistakes

- × Oversized tissue blocks → fixative cannot penetrate and the centre autolyses
- × Fixing before staining live-cell markers by flow → false negatives
- × Freezing samples without OCT → sections crack and crumble

Disclaimer: This guide provides general sample collection and handling recommendations to help improve sample quality and assay success. It does **not constitute medical, diagnostic, legal or regulatory advice**. Requirements may differ by sample type, species, matrix and project design; actual practice must follow the agreed project protocol, the signed Sample Collection Guide and written communication with the laboratory. Research involving clinical samples must comply with the ethics review and informed-consent requirements of your institution. We accept no indirect liability for failed assays, data deviation or sample loss caused by collection, storage or shipping that departs from this guidance. Content may be updated as methods evolve without further notice.

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