

FIMM HiPREP

Services and technologies for high-end sample preparations

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HiLIFE UNIT



The figures show sample flow and key variables in sample preparation including collection to handling, storage, extraction, quality and quantity control.

Samples & Preanalytics

Tissue

FFPE

- Sections, punches

Fresh frozen tissue

- Snap frozen
- RNAlater

Cells

Fresh frozen

- Cultured cells
- PBMC (CPT etc)
- Bone marrow cells
- Viable or non-viable

Blood

Blood

- Tempus, PaxGene
- Serum, plasma (EDTA, citrate, heparin)
- Fasting, non-fasting

Body fluids

Urine

- Urine (preservatives, e.g. protease inhibitors)
- 24h, ON, morning, spot

Freezing and storage

- LN2, -80°C, -20°C
- +4 °C, RT
- Time to preservation

Sectioning

- Section thickness
- Sectioning temperature

Centrifugation and ultracentrifugation

- Removal of contaminants
- Concentration
- G-value, K-factor, volume, temperature

Extraction

cfDNA

gDNA

mRNA

Small RNA

EV and EV-RNA

Homogenization

- Bead beating, mortar & pestle, needle, vortex, shaking, enzymatic treatment
- Strength, duration, temperature

RNA or DNA extraction or coextraction

- Magnetic bead or silica column
- Automated or manual
- Nucleic acid inclusion/removal
- Elution buffer and volume

- ### MOST IMPORTANT DEVELOPMENT ITEMS
- Multi-analyte extractions
 - High throughput protocols for clean-up and concentration targeting small final volumes
 - Quality control for emerging research areas
 - cfRNA, cfDNA
 - EV-RNA
 - FFPE RNA and DNA
 - Small RNA

Quality control & normalization

Tissue cryo-sections RNA
RIN 9.3

FFPE sections RNA
DV200 >50%

Plasma %cfDNA
80%

Urine EV-RNA
RIN: 2.80

Whole blood, Tempus RNA
RINe 8.2

Whole blood, Tempus DNA
DIN 9.0

Fragment size analysis

- RIN - RNA integrity
- DIN - gDNA integrity
- DV200 - FFPE RNA integrity
- %cfDNA – cfDNA proportion of total DNA

Concentration and contamination

- DNA, RNA, DNA/RNA-%, RNA/DNA-%
- A260/A280, A260/A230
- smallRNA, dsDNA, ssDNA
- Fluorometric methods (Qubit/Quant-It), Absorbance (Nanodrop), Fragment analysis (Bioanalyzer, TapeStation)

Normalization and extras

- Constant concentration or quantity
- DNase and RNase treatments
- Clean-up and concentration
- Buffer exchange

Electron microscopy

- EV morphology, size
- Purity, contaminants

HiPREP