RNA-Seq Express
Sample Preparation

ACTIVE MOTIF®
Enabling Epigenetics Research

Customers may submit cells, animal tissues or purified total RNA for our Express RNA-Seq Service (Illumina-based sequencing).

Input Type	Recommended Input	Minimun Requirement	Why Recommended?
Total RNA	Up to 1 µg	500 ng	Improves library complexity, transcript detection sensitivity, and overall data robustness
Mammalian Cells	50,000–100,000 cells	25,000–50,000 cells	Provides sufficient RNA yield for optimal sequencing performance
Fresh Frozen Tissue	≥50 mg	≥20 mg	Ensures adequate RNA recovery and consistent library quality

Protocol

Prepare frozen pellets from cell cultures

1. Culture cells under appropriate conditions and harvest 2 × 10^6 cells per sample. A minimum input of 2.5 × 10^4 cells is acceptable if necessary
2. Transfer the cell suspension to a prechilled 15 mL conical tube. For adherent cells, detach cells by scraping in cold PBS or by trypsinization prior to transfer.
3. Centrifuge at 800 × g for 5 minutes at 4°C to pellet the cells. Carefully aspirate and discard the culture medium/PBS.
4. Resuspend the cell pellet in 1 mL ice-cold PBS by gentle pipetting. Transfer the suspension to a prechilled 1.5 mL microcentrifuge tube and centrifuge again at 800 × g for 5 minutes at 4°C.
5. Carefully remove and discard the PBS. Snap-freeze the cell pellet in liquid nitrogen or on dry ice.
6. Store frozen cell pellets at -80°C until further processing.

Tissue Collection and Freezing

1. Excise the appropriate amount of tissue (approximately 50-100 mg for most tissue types). Record the exact weight of each sample on the sample submission form.
2. Transfer the tissue to a prechilled 1.5 mL microcentrifuge tube or a 5 mL tube and snap-freeze on dry ice.
3. Store frozen tissue samples at -80°C until RNA extraction.

Total RNA Preparation

Note: For adherent cells, add lysis buffer directly to the culture plate to maximize RNA recovery.

1. Isolate total RNA from cultured cells or animal tissue using a column-based RNA purification kit (e.g., Qiagen RNeasy Mini or Midi Kit) or an equivalent validated method.
2. Elute or resuspend RNA in nuclease-free water and determine concentration with Qubit and the total yield. Submit 0.5-3 µg of total RNA at a concentration of 10-50 ng/µL.
3. Store purified RNA at -80°C until shipment or further processing.