ALITHEA GENOMICS

MERCURIUS™

Cell Lysate BRB-seq Service

RNA Extraction-free protocol

Sample Submission Guidelines

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Sample Submission Guidelines at a glance

1. Lyse the cells in a 96-well plate following the instructions and using the provided reagents, transfer the lysates to a new PCR plate, and store it at -80°C before shipment.
2. Fill in the Sample Submission Form and send it to **info@alitheagenomics.com**
3. Ship the samples on dry ice and send us the shipment tracking number.

Consumables provided

Component Name	Label	Volume	Storage
Cell Lysis Buffer	CLB	355 µL	-20°C
RNase Inhibitor	INH	95 µL	-20°C
96-well PCR plate	-	-	RT
Aluminum seal for 96-well plate (Aluseal)	-	-	RT

Consumables not provided

Reagents	Manufacturer	PN
DPBS, no calcium, no magnesium	Gibco	14190144
Nuclease-free Water	Thermo	AM9937

Essential considerations for input material

- The recommended input range of cells is 10-50k/well of a 96-well plate.
- Cells must be seeded a few days in advance for the best results.
- Depending on the type of cells (human, mouse, cancer, or primary) and experimental design (e.g., drug treatment, induction of apoptosis, cell cycle arrest, etc.), consider the cell doubling time and the potential effect of the treatment on cell quality and quantity.
- To ensure an even distribution of reads after sequencing, the amount of starting material must be as uniform as possible. For this, we suggest using automatized cell seeding instruments or double-verified cell count.

Cell Lysis procedure

Cell Lysis Buffer (CLB) preparation

Prepare a working solution of Cell Lysis Buffer as follows:

Reagent	Per well (µL)	96 wells +10% (µL)
CLB	3.3	350
INH	0.83	90
NFW	5.9	664
TOTAL	**10**	**1104**

Pipette the prepared mix gently a few times, and briefly spin the tube. Keep the mix on ice until further use.

Adherent cells preparation

1. Seed the cells in a flat bottom 96-well plate at a density that will enable to harvest 10-50k cells per well.
2. Gently aspirate culture media from the plate and wash cells by adding 80-100 µL of DPBS in each well.
3. Gently tap the plate and aspirate as much DPBS as possible without disturbing the cell pellet.
4. Immediately proceed to step 11.

Cells suspension preparation

5. Seed the cells in a flat bottom or U-shaped 96-well plate at a density that will enable to harvest 10-50k cells per well.
6. Centrifuge the plate at 300x g for 5 min.
7. Gently aspirate culture media from the plate and wash cells by adding 80-100 µL DPBS in each well.
8. Centrifuge the plate at 300x g for 5 min.
9. Aspirate as much DPBS as possible without disturbing the cell pellet.
10. Immediately proceed to step 11.

Cell lysis

11. Using a multi dispenser, distribute 10 µL of prepared CLB in each well with pre-washed cells.
12. Gently tap the plate to ensure that CLB is uniformly distributed on the surface of each well.
13. Incubate the plate on ice for 15 min, slightly agitating it from time to time.
14. Label the provided 96-well PCR plate.
15. Transfer the entire volume of cell lysates from every well to the corresponding well of the new 96-well PCR plate. Preferably use a multichannel pipette and avoid transferring any cell pellet.
16. Seal the 96-well PCR plate with the provided Aluseal and briefly spin it down.
17. Store the lysates at -80°C before shipment.

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