ALITHEA GENOMICS

MERCURIUS™

DRUG-seq Service

Lysate Preparation and Sample Submission Guidelines

RNA Extraction-free Protocol for 96-well Plate Format

v.0.2.3. April 2024

DRUG-seq Service for 96-well Plate Format

Sample submission guidelines at a glance

1. Transfer the lysates into a 96-well PCR plate following the instructions below and store it at -80°C before shipment.
2. Fill in the Sample Submission Form **(SSF)** and **check all the boxes** in the Sample Submission Checklist below; send both files to **orders@alitheagenomics.com**. Please be aware that any inconsistency may lead to delays or additional fees.
3. Ship the samples on dry ice and send us the shipment tracking number.

   For samples in a 384-well plate format, contact **orders@alitheagenomics.com** for another version of the Service guidelines.

Sample Submission Checklist

- ☐ **Sample Submission Form (SSF)** is filled properly with a **unique sample ID**. Consider adding a suffix for technical replicates (e.g., XX_rep1, XX_rep2, etc.). Make sure that the information about **all the shipped samples is provided in the SSF**.
- ☐ The minimum sample volume is **10 µL** per well.
- ☐ The **minimum number** of samples in each group (to be pooled together) is **16**.
- ☐ Samples are provided in the 96-well PCR plates. **Samples in tubes cannot be processed.**
- ☐ Sample plates are clearly labeled with the same Plate ID as in the Sample Submission Form.
- ☐ Plates are well sealed with the provided Aluminum seal.

Consumables provided

Component Name	Label	Per plate +10%	Storage
Cell Lysis Buffer	CLB	710 µL	-20°C
RNase Inhibitor	INH	175 µL	-20°C
96-well PCR plate	-	2 plates	-20°C
Aluseal, aluminum seal for PCR plate	-	1 pc	RT

Another required consumables (not provided)

Reagents	Manufacturer	PN
DPBS, no calcium, no magnesium	Gibco	14190144
Nuclease-free Water	Thermo	A57775

Essential considerations for input material

- The recommended input range of cells is 15'000-50'000/well of a 96-well plate. However, this number can vary, depending on the cell type, cell size, and the mortality/viability of cells after the treatment.
- For the best protocol performance, the cells should be 90% confluent on the day of lysis.
- 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 the experimental design (e.g., drug treatment, induction of apoptosis, cell cycle arrest, etc.), consider the cell doubling time subsequent to seeding and the potential effect of the treatment on cell quality and quantity.
- The amount of starting material must be as uniform as possible to ensure an even distribution of reads after sequencing. For this, we suggest using automatized cell seeding instruments or double-verified cell counts.
- It is essential to respect the timing of the lysis procedure.

Cell Lysis Protocol

NOTE: Briefly spin down the tubes and plates before opening them to ensure that all liquid or particles are collected at the bottom of the tube/plate.

The working solution of 1x Cell Lysis Buffer consists of the following:

Reagent	Volume (µL)	
	Per well	96 wells +10%
CLB	6.6	700
INH	1.6	170
Nuclease-free water	11.8	1250
TOTAL	**20**	**2120**

Cell Lysis Buffer (CLB) preparation

1. Thaw the CLB tube on ice and avoid its long-term storage.
2. Keep the nuclease-free water on ice to maintain a cold temperature.
3. Spin down all the tubes before pipetting.
4. In a 15 mL falcon tube, add the water first, then the CLB, and the INH (in this particular order).
5. Pipette the prepared mix a few times and briefly spin the tube. Keep it on ice until further use.

Procedure for the preparation of adherent cells

1. Seed the cells in a flat bottom 96-well plate at a density that will enable harvesting 15'000-50'000 cells per well.
2. On the day of cell lysate preparation, gently aspirate the culture media from the plate and wash cells by adding 80-100 µL per well of room-temperature DPBS.
3. Gently tap the plate and aspirate as much DPBS as possible without disturbing the cells.
4. Immediately proceed to **Cell lysis procedure.**

Procedure for the preparation of suspension cells

1. Seed the cells in a flat bottom or U-shaped 96-well plate at a density that will enable harvesting 15'000-50'000 cells per well.
2. On the day of cell lysate preparation, centrifuge the plate at 300x g for 5 min.
3. Gently aspirate the culture media from the plate and wash cells by adding 80-100 µL per well of room-temperature DPBS. Centrifuge the plate at 300x g for 5 min.
4. Aspirate as much DPBS as possible without disturbing the cells.
5. Immediately proceed to **Cell lysis procedure.**

Cell lysis procedure

1. Using a multi dispenser, distribute 20 µL/well of prepared 1x CLB.
2. Gently tap the plate to ensure that CLB is uniformly distributed on the surface of each well.
3. Incubate the plate on ice for 15 min, slightly agitating it from time to time.
4. Label the provided 96-well plate.
5. To avoid lysate contamination with floating debris and nuclei, and sample contamination with gDNA, we **strongly recommend centrifuging the plate with lysates at 600x g for 5 min** (pre-cooled, +4°C).
6. 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.
7. Seal the 96-well PCR plate with the provided Aluseal and briefly spin it down.
8. Store the cleared lysates at -80°C before shipment.

NOTE: If several plates must be processed, perform the procedure individually per plate to avoid keeping the plates at room temperature for a prolonged time.

Alithea Genomics SA

Phone +41 78 830 31 39

Email info@alitheagenomics.com

Web www.alitheagenomics.com

Address
Route de la Corniche, 8
1066 Épalinges
VD, Switzerland