ALITHEA GENOMICS

MERCURIUS™

Full Length DRUG-seq (mRNA) Service

Sample Preparation and Submission Guidelines

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FL DRUG-seq Service for 96-well Plate Format

Sample submission guidelines at a glance

1. Wash the cells and store them in the original culturing plates 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.

Sample submission checklist

- ☐ The Sample Submission Form (SSF) must be filled out properly with a unique sample ID. Consider adding a suffix for technical replicates (e.g., XX_rep1, XX_rep2, etc.). Ensure that the SSF provides information about all the shipped samples.
- ☐ The **minimum number** of samples in each group (to be pooled together) is **16**.
- ☐ Sample plates are labeled with the same Plate ID as in the Sample Submission Form.
- ☐ Plates are well sealed with the Aluminum or plastic seal.

Required consumables (not provided)

Reagents	Manufacturer	PN
DPBS, no calcium, no magnesium	Gibco	14190144
Aluseal, adhesive aluminum seal for the cell plate	Thermo	AB0626

1. Essential considerations for input material

1.1 The recommended input range of cells is 5'000-15'000 cells per well for a 96-well plate. However, this number can vary depending on the cell type, size, and mortality/viability of cells after the treatment.
1.2 For the best protocol performance, the cells should be 90% confluent on the day of cell collection.
1.3 Cells must be seeded a few days in advance for the best results.
1.4 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 after seeding and the potential effect of the treatment on cell quality and quantity.
1.5 The starting material must be uniform to ensure an even distribution of reads after sequencing. For this, we suggest automatizing cell seeding instruments or double-verified cell counts.

2. Cell Pellets Preparation Protocol

2.1. Procedure for the preparation of adherent cells

2.1.1 Seed the cells in a flat bottom 96-well plate at a density that will enable harvesting enough cells (see p.1.1).
2.1.2 On the day of cell pellet preparation, gently aspirate the culture media from the plate and wash the cells by adding 80-100 µL/well of a room-temperature DPBS.
2.1.3 Gently tap the plate and aspirate as much DPBS as possible without disturbing the cells.
2.1.4 Seal the plate well with an Aluseal and immediately transfer it to a -80°C freezer for storage. If possible, snap-freeze the plate with dry ice beforehand.

2.2. Procedure for the preparation of suspension cells

2.2.1. Seed the cells in a U-shaped 96-well plate at a density that will enable harvesting enough cells (see p.1.1).
2.2.2. On the day of cell pellet preparation, centrifuge the plate at 300x g for 5 min.
2.2.3. Gently aspirate the culture media from the plate and wash the cells by adding 80-100 µL/well of a room-temperature DPBS.
2.2.4. Gently tap the plate and centrifuge it at 300x g for 5 min. Aspirate as much DPBS as possible without disturbing the cells.
2.2.5. Seal the plate well with an Aluseal and immediately transfer it to a -80°C freezer for storage. If possible, snap-freeze the plate with dry ice beforehand.

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.

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