ALITHEA GENOMICS

MERCURIUS™

Total DRUG-seq Service

Sample Preparation and Submission Guidelines

Extraction-Free Protocol for
96- and 384-well Plate Format

February 2026

Total DRUG-seq for 96- and 384-well Plate Format

Sample submission guidelines at a glance

1. Prepare the samples as described and store them at -80°C prior to shipment.
2. Fill 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 inconsistencies may result in delays or additional fees.
3. Request the **shipping address** from your sales specialist.
4. Ship the samples on dry ice, ensuring the plates are placed between layers of dry ice to maintain a consistent freezing temperature throughout transit. Please provide us with the shipment tracking number.

Sample submission checklist

- ☐ Total DRUG-seq service is optimized for efficient rRNA depletion in **human, mouse,** and **rat** cells. For use with other species, please get in touch with us at **info@alitheagenomics.com** to discuss compatibility.
- ☐ The Sample Submission Form (SSF) must be filled out correctly 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.
- ☐ Avoid having randomly distributed samples across the plate layout (e.g., in A01, A06, B03, G10-12, etc.).
- ☐ The **minimum number** of samples in each group (to be pooled together) is **16** (for the 96-well plate) and **150** (for a 384-well plate).
- ☐ One type of seeded cell per well, with only one type of cell pooled together, and a minimum of 45’000 cells per pool.
- ☐ Plates are labeled with the same Plate ID as indicated in the SSF.
- ☐ Plates are well sealed with an adhesive and a temperature-resistant seal (aluminum is ideal).

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 cells

1.1 The recommended input range of cells is:
- 5'000-25'000 cells per well for a 96-well plate;
- 2'000-10'000 cells/well for a 384-well plate.

It is advisable to use a cell number closer to the lower end of the recommended range rather than the upper limit.

1.2 **NOTE:** Do not use more than 25'000 cells per well, as it will result in a high rRNA content.

1.3 Cells should be seeded a few days in advance for optimal results.

1.4 To obtain the best results before the experiment, ensure cell viability is >70% (e.g., trypan blue, propidium iodide.

1.5 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.

1.6 The starting material must be uniform to ensure an even distribution of reads after sequencing. For this, we suggest automating cell seeding instruments or double-verified cell counts.

2. Cell pellet preparation

2.1. Procedure for the preparation of adherent cells

2.1.1 Seed the cells in a flat-bottom 96- or 384-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 room-temperature DPBS:
- 80-120 µL per well (for 96-well plate);
- 30-50 µL per well (for 384-well plate).

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- or 384-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 room-temperature DPBS:
- 80-120 µL per well (for 96-well plate);
- 30-50 µL per well (for 384-well plate).

2.2.4. 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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