ALITHEA GENOMICS

MERCURIUS™

Spheroid DRUG-seq Service

Sample Preparation and Submission Guidelines

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

August 2025

(Early-Access)

Spheroid 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

- ☐ 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 the 384-well plate).
- ☐ One type of seeded spheroid per well, with only one kind of spheroid pooled together, and a minimum of 80’000 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 material

1.1. The spheroid lysate protocol was validated using spheroids cultured in U-shaped and microtissue-specific 96- or 384-well plates (e.g., Akura™, BIOFLOAT™).

1.2. The recommended input range of cells in spheroids on the day of harvesting is:

- **96WP** (U-bottom plate): 5'000-50'000 cells/well
- **96WP** (Microtissue-specific plate) and **384WP**: 2'000-10'000 cells/well

1.3. Spheroids should be plated a few days in advance for optimal results.

1.4. Depending on the type of spheroids (human, mouse, metastatic, or primary cells) and experimental design (e.g., drug treatment, induction of apoptosis, cell cycle arrest, etc.), consider the cell doubling time and potential effect of the treatment on cell quality and quantity.

1.5. 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 automated cell seeding instruments or double-verified cell counts.

2. Spheroid pellet preparation

2.1. Gently aspirate culture media from the plate and wash Spheroids by adding DPBS (per well):

- **96WP** (U-bottom plate): 80-100 µL DPBS
- **96WP** (Microtissue-specific plate) and **384WP**: 20µL DPBS

2.2. Centrifuge at 300x g for 3 min, if necessary.

2.3. Gently tap the plate and aspirate as much DPBS as possible without disturbing the spheroids' structure.

2.4. 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.

NOTE: If multiple plates must be processed, perform the procedure individually for each plate to avoid keeping them at room temperature for an extended period.