ALITHEA GENOMICS

MERCURIUS™

Organoid DRUG-seq Service (Frozen organoid pellet)

Sample Preparation and Submission Guidelines

RNA Extraction-free Protocol for 96- and 384-well Plate Format

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Sample Submission Guidelines at a glance

1. Wash the organoids 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 properly with a unique sample ID. Consider adding a suffix for technical replicates (e.g., XX_rep1, XX_rep2, etc.). Ensure that SSF provides information about all shipped samples.
- ☐ The **minimum number** of samples in each group (to be pooled together) is **16** (for 96-well plate) and **150** (for 384-well plate).
- ☐ One type of seeded cell per well and only one type to be pooled together, with a minimum of 80’000 cells per pool.
- ☐ 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.

Consumables not provided

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

1. Essential considerations for input material

1.1 The organoids lysate protocol was validated with organoids and spheroids, cultured in **flat-bottom 96-well plates with Matrigel®** or **microtissue-specific 96-well plates** (e.g., Akura™, BIOFLOAT™, ULA Corning plates, etc.)

1.2. The recommended input range of cells in organoids or spheroids is 5'000-50'000 cells/well of 96WP and 2'000-10'000 cells/well of 384WP.

1.3. Organoids must be plated a few days before for the best results.

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

2. Organoid Pellets Preparation for input material

2.1 Gently aspirate the culture media from the plate and wash organoids or spheroids by adding DPBS (per well):

- 100 µL for **96WP flat-bottom plates with Matrigel®**,
- 20 µL for **96WP microtissue-specific plates and 384WP**.

2.2 In case of spheroids, centrifuge plate at 300x g for 5 min.
2.3 Gently tap the plate and aspirate as much DPBS as possible without disturbing the structure of the organoids.
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.
2.5 Store the plate 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.

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