Element Biosciences

U2OS Cell Culture Protocol
For use with Cytos™ Cell Screening

Introduction

This document describes a protocol for culturing **U2OS** cells on a Cytos Slide Kit for a Cytos cell screening run using an AVITI24™ system. The protocol provides details on seeding freshly dissociated cells onto a treated Cytos Slide Kit for growth and proliferation to ensure cell growth as a consistent cell layer.

To achieve the optimal density for your experiment, perform seeding density optimization using Cytos Slide Kit and Cytos Onboard Optimization Kit with an AVITI24 system or using square, glass bottom plates (ibidi, catalog # 89627 or # 88407) with brightfield microscopy.

U2OS Cells

U2OS cells are adherent, immortalized human epithelial cells derived from a moderately differentiated osteosarcoma. Follow the manufacturer's instructions on thawing, subculturing, and maintaining U2OS cells. Use healthy cells that exhibit > 85% viability with trypan blue staining for the Cytos assay.

Additional Documentation

For detailed instructions on seeding density optimization, sample preparation guidelines, custom surface coatings, fixation, and permeabilization protocols, see Cytos Sample Preparation & Optimization User Guide (MA-00094).

Materials Needed

The following consumables are required for this protocol:

- Cytos Slide Kit coated with a preferred surface coating
- Cytos Centrifuge Adapter (part # 860-00069)
- U2OS cells (freshly dissociated)
- Prewarmed cell culture medium appropriate for the cell line
- Pipettes (8-channel or 16-channel for 48-well slide kits, 8-channel for 12-well slide kits, and single-channel for 1-well slide kits)
- [Optional] Plate rocker (VWR Scientific Platform Rocker, catalog # 444-0927) or shaker (Qinstruments Bioshake XP, catalog # 1808-0505)

Surface Coatings

Cytos Slide Kits are uncoated and require a custom surface coating compatible with the cell type. For U2OS cells, coat the Cytos Slide Kits with Poly-L-Lysine (PLL).

Follow the steps in **Custom Surface Coatings** section of the Cytos Sample Preparation & Optimization User Guide (MA-00094) for the surface coating protocol.

Culture U2OS Cells on a Cytos Slide Kit

Perform all steps in a biosafety cabinet.

1. Warm the cell culture medium in a 37°C water bath.
2. If your slide kit with custom surface coating was stored in liquid, remove any liquid in the wells.
3. Wash each well with the appropriate volume of prewarmed culture medium. Slightly tip the slide kit and slowly dispense along the wall of each well. Do not contact the slide surface. Ensure the media covers the surface in each well.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
50 µl	200 µl	3 ml

NOTE
For the 48-well slide kit, use a P-200 multichannel pipette or 16-channel Finnpipette.

4. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
5. Repeat the wash one more time.
6. Ensure the cells are fully dissociated to single cells and counted.
7. With the slide kit on a flat and level surface, hold the pipette perpendicular to the well and gently dispense the appropriate volume of suspended cells in the center of the well. Do not swirl the pipette when loading. Do not contact the slide surface.

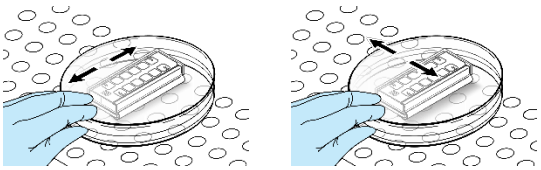
   The estimated initial cell seeding density is as follows:

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	150 µl	2 ml
4000 cells per well	13 K cells per well	173 K cells per well

8. Cover the slide kit. Distribute the cells manually or use a plate rocker or plate shaker as follows:

   Manual cell distribution

   - Gently distribute the cells for 30 seconds using a forward-and-back, then side-to-side motion.
   - At a slow pace, move forward and back and side to side covering at least 5 inches (12.5 cm) in each direction.
   - *Do not move in a circular motion.* Do not allow the liquid to splash within the wells.



   Using a Plate Rocker or Plate Shaker to Distribute Cells

   - Place the slide kit in a Cytos Centrifuge Adapter (part # 860-00069).
   - Set the adapter with slide kit on the shaker or rocker as follows:
     - Set the plater rocker at speed set at 2 and rock the slide kit for 2.5 minutes for cell distribution.
     - Set the plate shaker at speed set at 200 for 2.5 minutes for cell distribution.
   - Rotate the slide kit to 90° at ~ 1 minute to ensure proper cell distribution in all directions.

9. **For the 48-well slide kit only—**Place the slide kit in a Cytos Centrifuge Adapter (part # 860-00069) and centrifuge at 130 x g for 2.5 minutes. Balance the centrifuge with another adapter.
10. Place the slide kit in a 10 cm Petri dish and then place the Petri dish with the slide kit in the incubator. Incubate the cells at **37°C for 16 to 24 hours** to target ideal confluency of 50–70% in each well. Do not allow cell overgrowth.
11. Proceed to **Fix Cultured Adherent Cells** and continue with **Sample Preparation and Optimization** steps to prepare cell samples for a Cytos run as described in the Cytos Sample Preparation & Optimization User Guide (MA-00094).

Assess Cell Sample

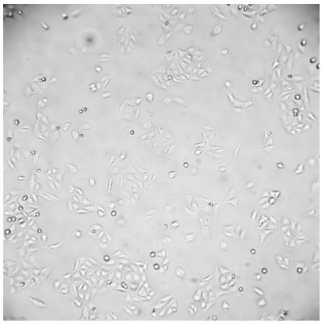
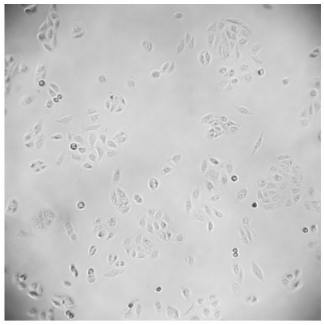
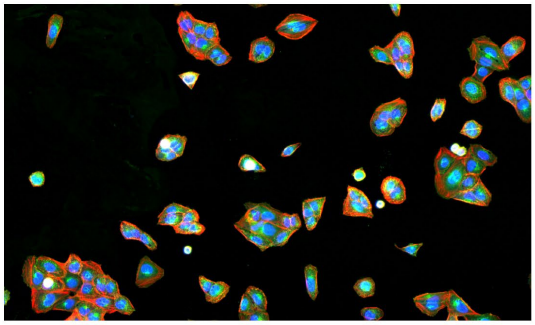
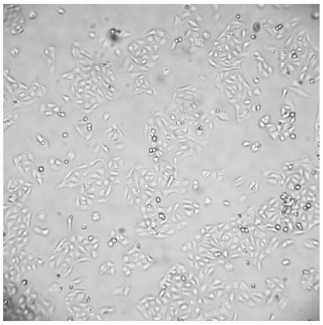
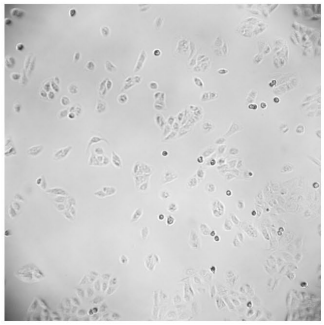
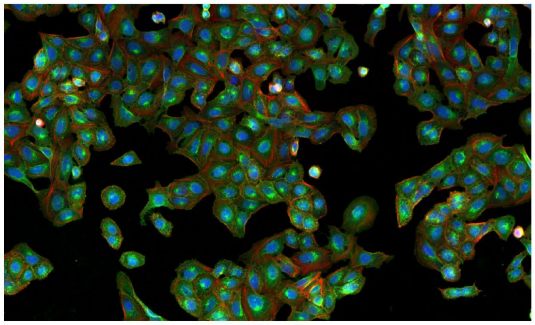
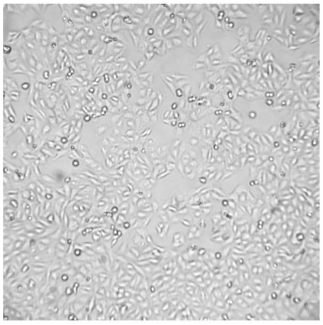
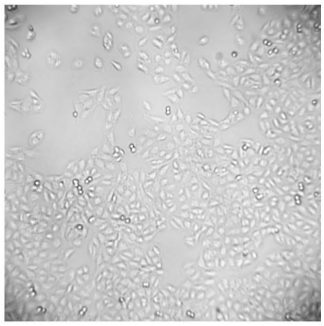
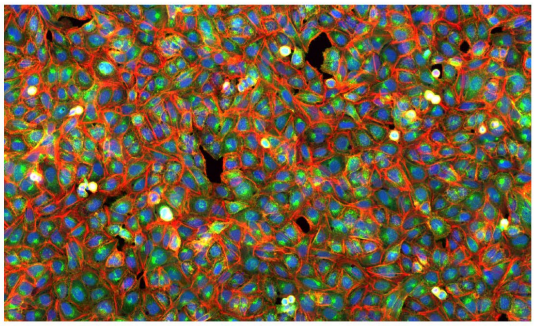
The following examples demonstrate successful cell culture technique and confluency for U2OS cells grown on a **48-well** Cytos Slide Kit and assessed by brightfield microscopy before and after fixation. The examples also show cell morphology and marker localization using the Cytos Onboard Optimization Kit with cell paint markers, imaged on the AVITI24 system and visualized using CytoCanvas™. On-instrument visualization of the cell paint allows you to assess the background and segmentation.

Visit the Cell Culture Hub to view brightfield microscopy images of seeding density experiments.

Reference the hub images to compare and assess if cells are under or over confluent, or at the ideal confluency.

Cell paint marker targets and display colors for AVITI24 images selected for this experiment are as follows:

- Wheat germ agglutinin (WGA): cell membrane marker, shown in green
- RedDot2: nuclear marker, shown in blue
- Phalloidin: actin marker, shown in red

Pre Fixation	Post Fixation	AVITI24 Cell Paint Images
		
Under Confluent: 2 K Cells	Under Confluent: 2 K Cells	Under Confluent: 2 K Cells
		
Ideal Confluency: 4 K Cells	Ideal Confluency: 4 K Cells	Ideal Confluency: 4 K Cells
		
Over Confluent: 8 K Cells	Over Confluent: 8 K Cells	Over Confluent: 8 K Cells

Note: Images are taken from different fields of view and may show a difference in cell density. Some cell types may show a 5–10% cell loss after fixation.

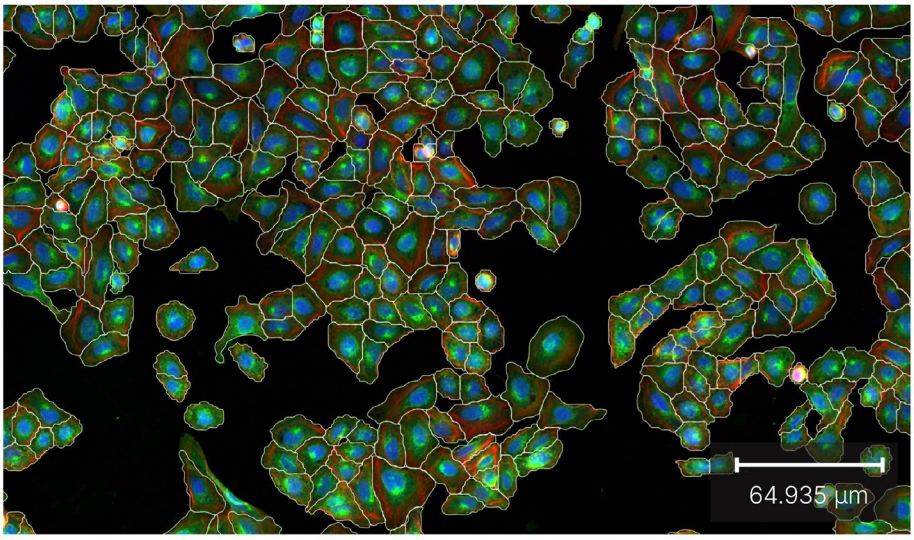
Segmentation of U2OS Cells

Segmentation of U2OS cells cultured on **48-well** Cytos Slide Kit assayed using the Cytos Onboard Optimization Kit with cell paint markers and imaged on the AVITI24 system was performed using the Element Biosciences™ pre-trained HeLa model.

The cells show ideal confluency and display good segmentation.

Cell paint marker targets and display colors for AVITI24 images selected for this experiment are as follows:

- WGA: cell membrane marker, shown in green
- RedDot2: nuclear marker, shown in blue
- Phalloidin: actin marker, shown in red



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