

Jurkat Cell Culture Protocol

For use with Cytos™ Cell Screening

Introduction

This document describes a protocol for culturing **Jurkat** cells on a Cytos Slide Kit for a Cytos cell screening run using an AVITI24™ system. The protocol provides details on attaching and immobilizing live suspension cells onto a treated Cytos Slide Kits using centrifugation.

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.

Jurkat Cells

Jurkat cells are suspension, immortalized human T lymphocyte cells derived from T-cell lymphoblastic leukemia. Follow the manufacturer's instructions on thawing, subculturing, and maintaining Jurkat 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)
- Jurkat cells (freshly dissociated)
- 1X Dulbecco's Phosphate Buffered Saline (DPBS), with calcium, magnesium, pH 7–7.4
- 15 ml or 50 ml Falcon tube
- 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 Jurkat 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.

Attach Jurkat Cells on a Cytos Slide Kit

Perform all steps in a biosafety cabinet.

1. Warm the DPBS 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. Centrifuge the cells at **300 x g for 5 minutes** in a 15 ml or 50 ml Falcon tube depending on final volume.
4. Remove the supernatant without disturbing the cell pellet.
5. Add 1X DPBS to resuspend the cell pellet and dilute the cell solution depending on desired confluency.
6. With the slide kit on a flat and level surface, hold the pipette perpendicular to the well and gently dispense the appropriate volume of cell solution in the center of the well. Do not swirl the pipette when loading. Do not contact the slide surface.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	150 µl	1.5 ml
25–35 K cells per well	150 K cells per well	2 M cells per well

NOTE

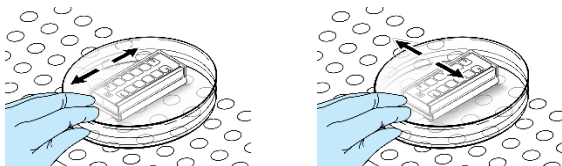
For the 48-well slide kit, use a P-200 multichannel pipette or 16-channel Finnpipette.

7. Cover the wells with the slide kit lid.
8. Depending on your slide kit, proceed to Step 8a or 8b.
 - a. For **48-Well or 12-Well Cytos Slide Kit**, load the covered slide kit onto a Cytos Centrifuge Adapter (part # 860-00069)
 - If preparing only one slide kit, load it in the center position.
 - Balance the centrifuge with another slide kit tray.
 - If preparing more than one slide kit, divide the slide kits between two slide kit trays.
 - Centrifuge at **300 x g for 15 minutes**.

- b. For **1-Well Cytos 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 plate 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.
 - Incubate the 1-Well Cytos Slide Kit at **room temperature for 15 minutes**.
 - Load the covered 1-Well Cytos Slide Kit onto a Cytos Centrifuge Adapter (part # 860-00069). Load only one slide in the center position and balance the centrifuge with another slide kit tray.
 - Centrifuge at **20 x g for 2 minutes**.
9. 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

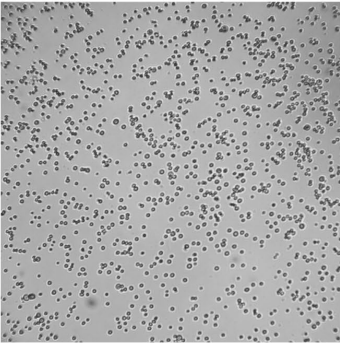
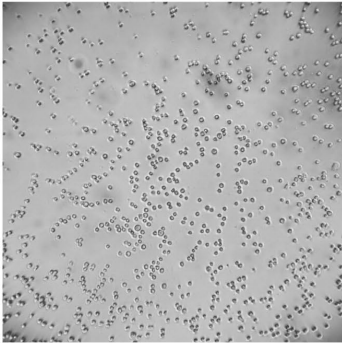
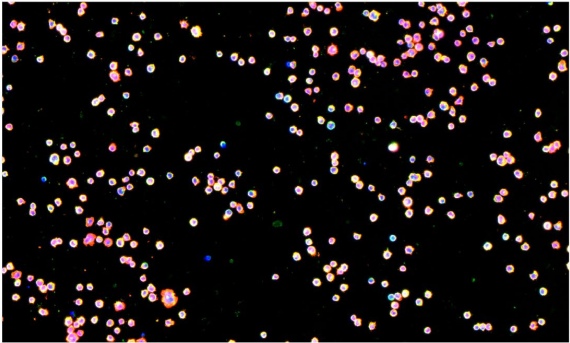
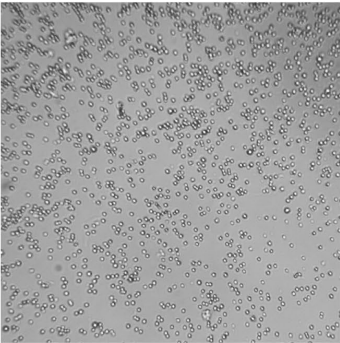
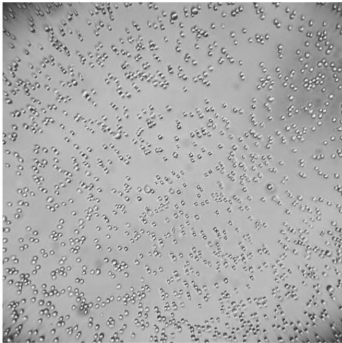
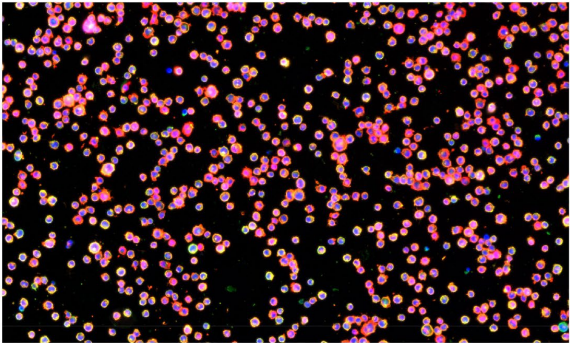
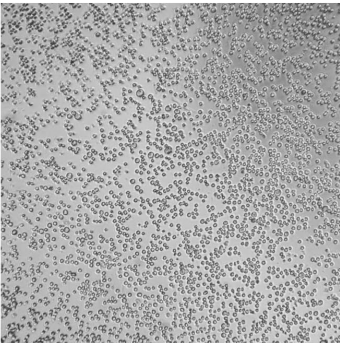
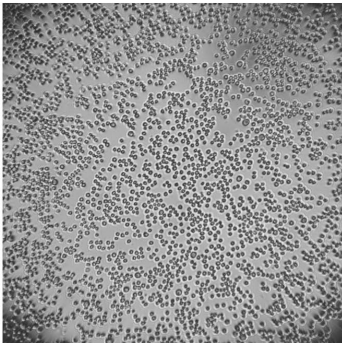
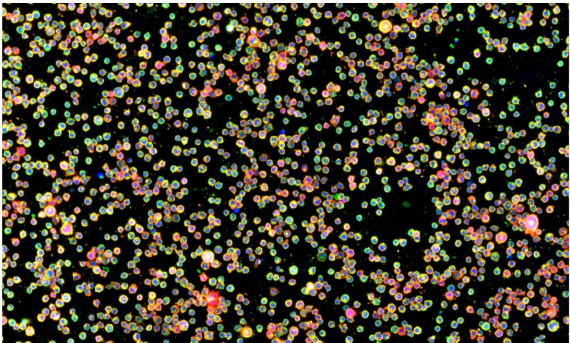
The following examples demonstrate successful cell culture technique and confluency for Jurkat cells grown on a **48-well** Cytos Slide Kit and assessed by brightfield microscopy before and after fixation. The examples also show cell morphology and marker localization using the Cytos Onboard Optimization 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 for Jurkat cells.

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: 12.5 K Cells	Under Confluent: 12.5 K Cells	Under Confluent: 12.5 K Cells
		
Ideal Confluency: 25 K Cells	Ideal Confluency: 25 K Cells	Ideal Confluency: 25 K Cells
		
Over Confluent: 50 K Cells	Over Confluent: 50 K Cells	Over Confluent: 50 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.

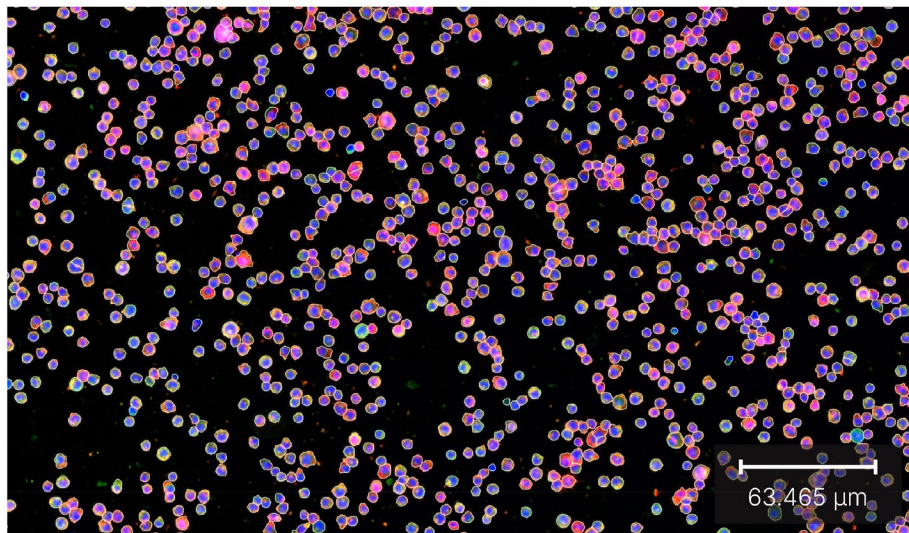
Segmentation of Jurkat Cells

Segmentation of Jurkat 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 Jurkat 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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