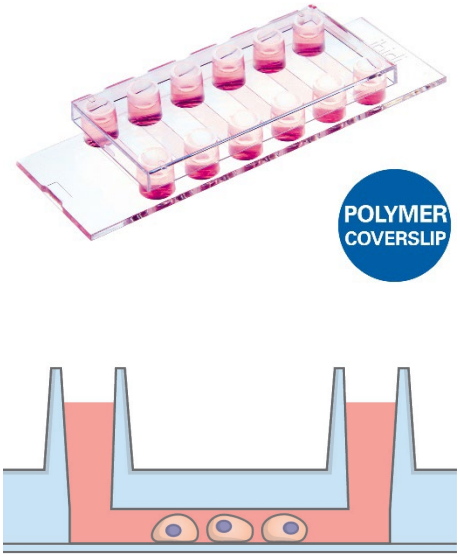
Cultivation and Detachment of Adherent Cells in the µ-Slide VI 0.4



The ibidi µ-Slide VI 0.4 is a versatile tool in cell culture and microscopy, known for its ability to support homogeneous cell distribution across its six channels. This slide is particularly efficient for immuno-fluorescence and live cell imaging assays, making it an invaluable asset for detailed visualization of cellular structures such as the actin cytoskeleton and mitochondria. Its low-volume design facilitates cost-effective experiments with minimal cell and reagent amounts and is compatible with both static and flow conditions.

This protocol focuses on the optimal handling technique of the µ-Slide VI 0.4 during cell seeding, medium exchange, and splitting of adherent HT-1080 cells, tailored to the slide's unique features. This protocol employs Accutase for detaching cells. However, depending on your experimental needs, you can also use trypsin instead.

For more insights on the benefits of using the µ-Slide VI 0.4, take a look at Application Note 03 "Cell Culture in ibidi Channel Slides" (PDF).

1 Materials

1.1 Reagents and Buffers

- HT-1080 cells (85111505, Sigma Aldrich)
- Cell culture medium: DMEM (D6546, Sigma Aldrich) with 10% Fetal Bovine Serum (F1283, Sigma Aldrich)
- D-PBS (14190144, Gibco)
- Accutase (A1110501, Gibco)

1.2 Equipment

- µ-Slide VI 0.4 ibiTreat (80606, ibidi)
- µ-Slide Rack (80003, ibidi)
- Standard cell culture equipment (pipettes, sterile working bench, cell culture incubator, culture flasks, hemocytometer, etc.)
- Phase contrast microscope

2 Methods

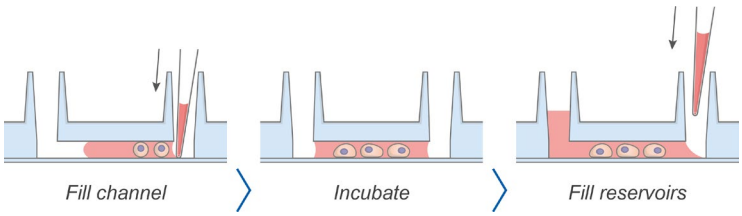
2.1 Cell Seeding

Please read the instructions before working with the µ-Slide VI 0.4. Perform all steps under sterile conditions. It is recommended to put the µ-Slide VI 0.4 and the cell culture medium into the incubator the day before seeding the cells to avoid the formation of air bubbles during handling. Before starting the experiment, prepare the HT-1080 cells in a standard cell culture flask (e.g., T75) with adherent cells at the bottom. The cells should ideally be subconfluent and healthy on the day of the experiment.

It is essential to work swiftly during the whole procedure to prevent the cells from drying.

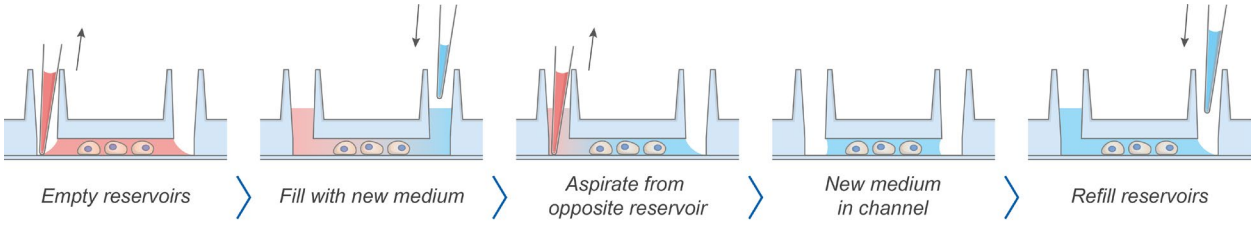
If not stated otherwise, all given volumes are per channel.

- Add 10 ml Accutase to the T75 flask for cell detachment; incubate for 5 min in the incubator (37°C, 5% CO_2).
- Stop the Accutase reaction by adding an equal volume of cell culture medium.
- Harvest the cell suspension, centrifuge, and dilute it in a low amount of cell culture medium for counting.
- Count the cells and adjust to a final concentration of 3×10^5 cells/ml in cell culture medium.
- Unpack an ibidi µ-Slide VI 0.4 and put it on a µ-Slide Rack or an appropriate surface.
- Pipet 30 µl of the HT-1080 cell suspension directly into each channel. Quick dispensing and pointing the tip towards the channel upon filling helps to avoid trapped air bubbles. Remove any trapped air bubbles from the channel by inclining the µ-Slide and tapping one edge.
- Cover the reservoirs with the supplied lid.
- Put the µ-Slide with the rack into the incubator (37°C, 5% CO_2) and let the cells attach for 1 h.
- Fill 60 µl cell culture medium into each reservoir. Do not trap air bubbles.
- Put the µ-Slide with the rack into the incubator (37°C, 5% CO_2) and incubate the cells overnight.
- For extended cell cultivation, we recommend a continuous medium exchange every second day (see Chapter 2.2).



2.2 Continuous Medium Exchange

- Carefully aspirate the medium from the reservoirs. Do not aspirate any liquid from the channel itself. Pointing the tip away from the channel helps.
- Gently introduce 120 µl of cell culture medium into one reservoir, replenishing the channel.
- Aspirate the old cell culture medium from the opposite reservoir. Be careful when using a cell culture aspiration device, as this may flush away partially attached cells or clusters. Pointing the tip away from the channel helps.
- Refill the reservoirs using 60 µl cell culture medium per reservoir.



2.3 Cell Detachment

- Carefully replace the cell culture medium with D-PBS through continuous medium exchange for washing.
- Carefully aspirate the entire channel volume.
- Immediately refill the channel with 30 µl of Accutase by placing the pipet tip directly on the channel's inlet.
- Incubate cells for 5 min at 37°C.
- Verify cell detachment using a phase contrast microscope. Cell detachment can be recognized by cell rounding; if cells are still attached, continue incubating for a few minutes.
- Flush each channel with 100 µl cell culture medium to stop cell detachment.
- Aspirate the cell suspension from the opposite reservoir; if any cells remain, repeat the flushing step.
- Centrifuge the cell suspension, remove the supernatant, and proceed with the next steps.