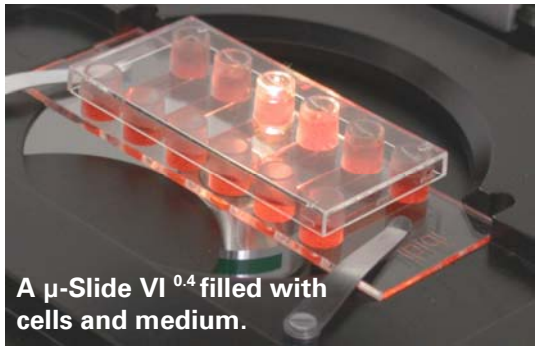
Trypsinization of adherent cells using µ-Slide VI 0.4

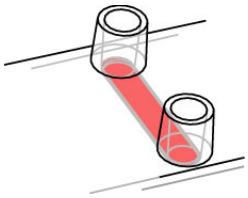
In this application note we show how to remove adherent cells from a µ-Slide VI $^{0.4}$ after cultivation. This protocol may be adapted to different channel format µ-Slides.

1. Grow your cells to the desired degree of confluence according to the instructions on www.ibidi.com.

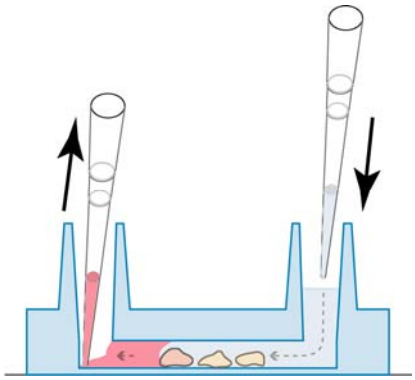


A µ-Slide VI $^{0.4}$ filled with cells and medium.

2. Remove the culture medium from the reservoirs of the µ-Slide VI $^{0.4}$. Do not aspirate the entire channel volume.

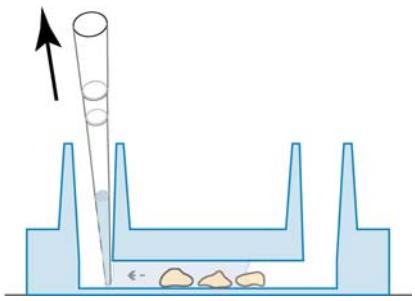


3. Wash twice with PBS or any other appropriate buffer and aspirate from the opposite end. Use a volume of 100 µl per channel. We recommend using a cell culture aspirator and a pipette at the same time. Use the tip of the aspirator and the pipet as illustrated.



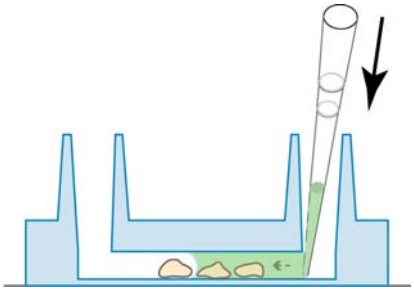
Cross section of one channel of the µ-Slide VI $^{0.4}$ showing the PBS washing step.

4. Aspirate the entire PBS from the channel by using the cell culture aspirator.



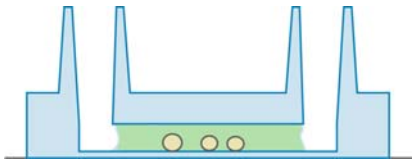
In this step the buffer is removed completely from the reservoirs and the channel.

5. Refill the channel with 30 µl of your detachment solution (e.g. Trypsin/ EDTA or Accutase) right away. Put the pipet tip directly on the channel's inlet as shown.



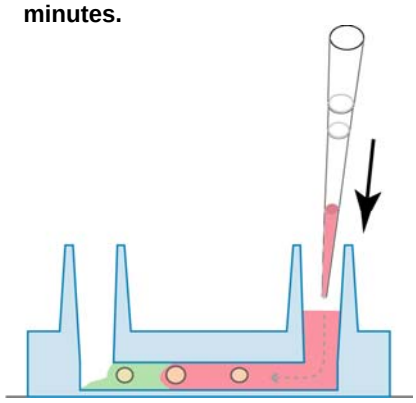
30 µl of detachment solution is filled into the empty channel.

6. Put your cells into the incubator. Due to the different aspect ratio of growth area and volume, the detachment process might take longer than usual (~2-3 min). Control cell detachment with the phase contrast microscope. If no detachment occurs, increase the concentration of your detachment solution or use a longer incubation time.



The cells will detach after some minutes.

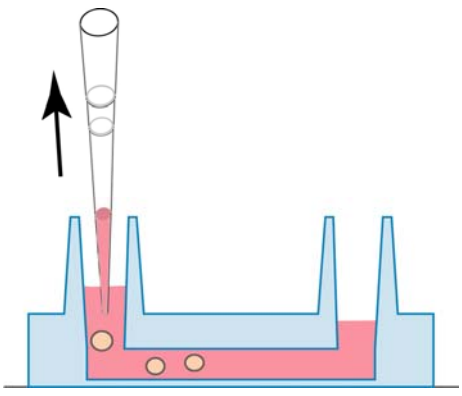
7. After cells are round and detached, flush each channel with 100 µl fresh medium or stopping solution.

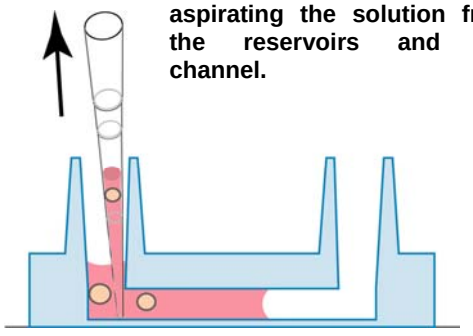


The detached cells are flushed out of the channel by 100 µl fresh medium.

8. Take out the cell suspension from the opposite end of the channel. In case there are some cells left, repeat the flushing step.

9. Collect the suspended cells and remove or dilute the detachment solution.





After the cells are suspended they can be collected by aspirating the solution from the reservoirs and the channel.