

Cultivation of Macrophages in μ -Slides VI^{0.4} and 8 well



1. General information

The following application note describes the cultivation protocol for the murine macrophage cell line **RAW-264.7** (Biosafety Level 2!), in the ibidi μ -Slide VI^{0.4} and μ -Slide 8 well. It is an example for a special cell line as a demonstration for adherence time, cell densities and cell morphology. This protocol can be adapted for your special experimental demands.

2. Material

In the setup the following material is applied:

- RAW-264.7 (murine macrophages, CLS - Cell Lines Service, Biosafety Level 2)
- Medium: RPMI 1640 with 2 mM L-Glutamin and 10% FBS
- μ -Slide VI^{0.4}, ibiTreat
- μ -Slide 8 well, ibiTreat
- Accutase (PAA Laboratories GmbH)
- 1x Phosphate buffered saline (PBS)

3. Cell culture and preparation of the material

Before seeding cells into the μ -Slides cultivate your cells according to your normal protocol.

μ -Slide VI^{0.4}: Equilibrate slides and medium over night inside the incubator at 37°C and 5% CO₂. This is essential to avoid air bubbles emerging over time. For this fill the appropriate amount of medium in a 50 ml vessel and leave the cap slightly screwed on. In an open form such as μ -Slide 8 well air bubbles can escape to the atmosphere.

Unpack the μ -Slide, place it on a μ -Slide rack and put the caps on the Luer adapters/wells respectively while preparing the cell suspension.

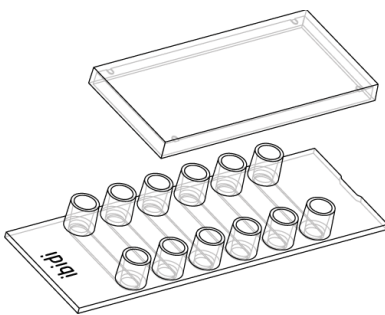
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4. Seeding cells

Detaching the cells:

Aspirate the medium of your cultivation flask and wash the cells once with PBS. Add 3-5 ml of Accutase per 75 cm² flask, and put the flask in the incubator for a faster detaching. In case of the RAW-264.7 cell line this takes at about eight minutes. The use of Accutase leads to a much higher viability of the detached cells.

4.1. Seeding cells into μ -Slide VI^{0.4}



Recommended cell concentration for μ -Slide VI^{0.4}:

For a final cell number of 0.3×10^5 cells/cm² prepare a concentration of 6×10^5 cells/ml. Fill 30 μ l of the cell suspension into every channel by putting the pipet tip directly onto the channel's inlet. Put the lid on the slide and incubate half an hour at 37°C and 5% CO₂ for cell attachment.

After cell attachment, fill the reservoirs with 60 μ l cell-free medium each. Avoid pointing the pipet tip directly onto the channel's inlet. Put the slide back in the incubator.

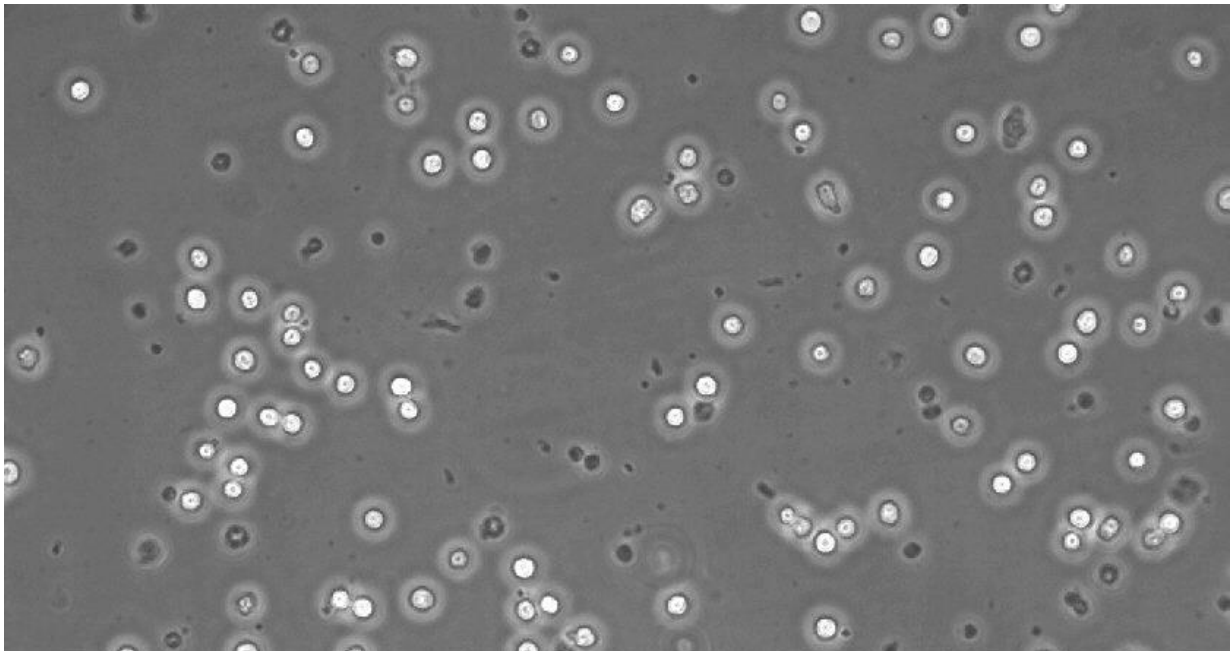


Fig. 1: RAW-264.7 in a μ -Slide VI^{0.4}, ibiTreat, one hour after seeding.

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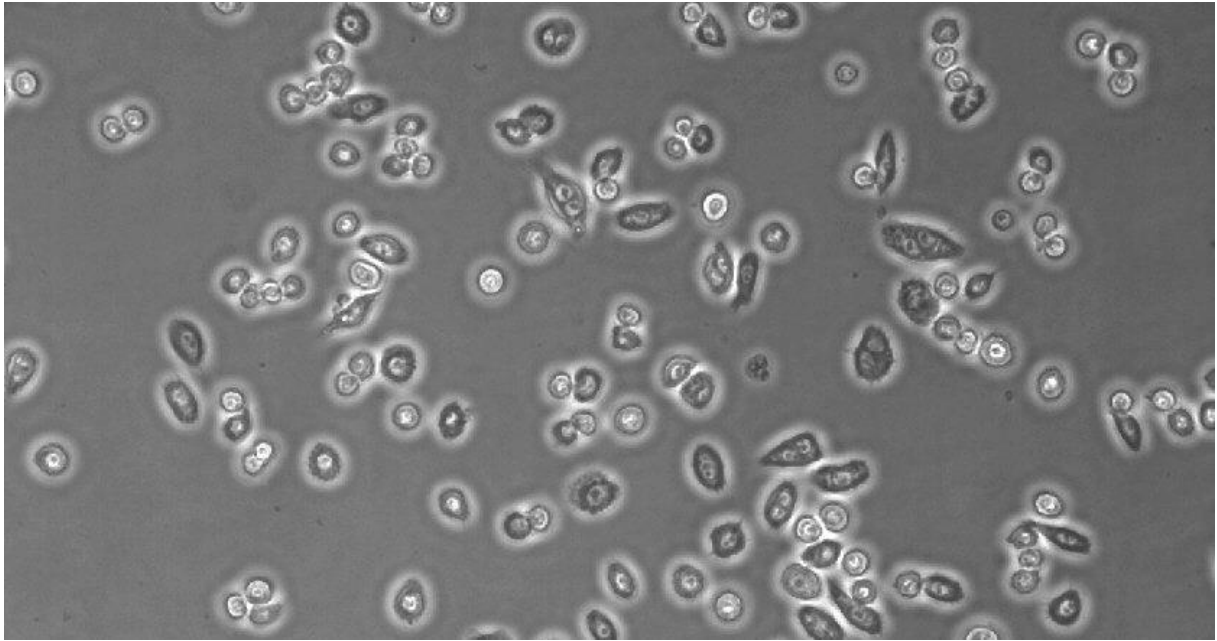


Fig.2: RAW-264.7 in a μ -Slide VI^{0.4}, ibiTreat, after 24 hours incubation.

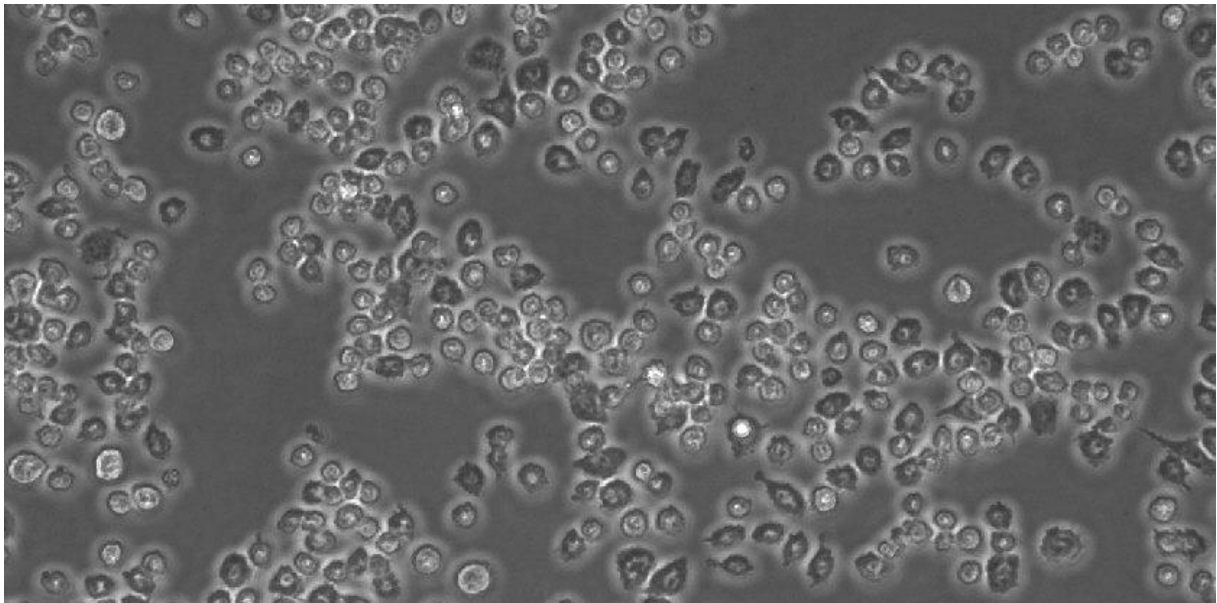
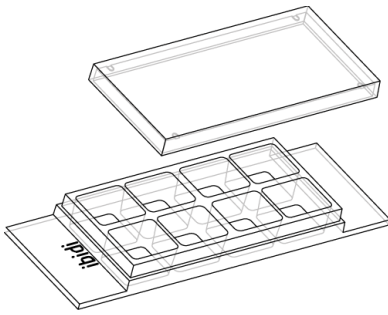


Fig.3: RAW-264.7 in a μ -Slide VI^{0.4}, ibiTreat, after four days of cultivation.

For more detailed information about cell culture in μ -Slide VI^{0.4} please also refer to the instructions on www.ibidi.com and to the [Application Note 3 \(Growing cells in \$\mu\$ -channels\)](#).

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4.2. Seeding cells into μ -Slide 8 well



Recommended cell concentration for **μ -Slide 8 well**:

For a final cell number of 0.3×10^5 cells/cm² prepare a concentration of 1.1×10^5 cells/ml. Fill 300 μ l of the cell suspension into every well. Put the lid on the slide and incubate at 37°C and 5% CO₂. No further addition of medium is necessary.

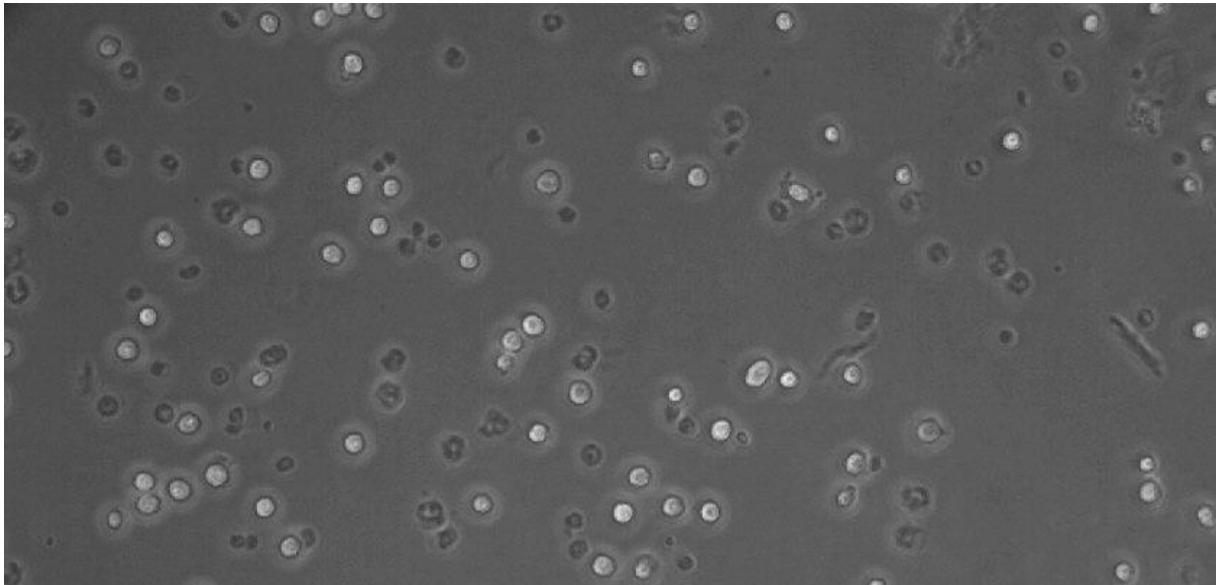


Fig. 4: RAW-264.7 in a μ -Slide 8 well, ibiTreat, one hour after seeding.

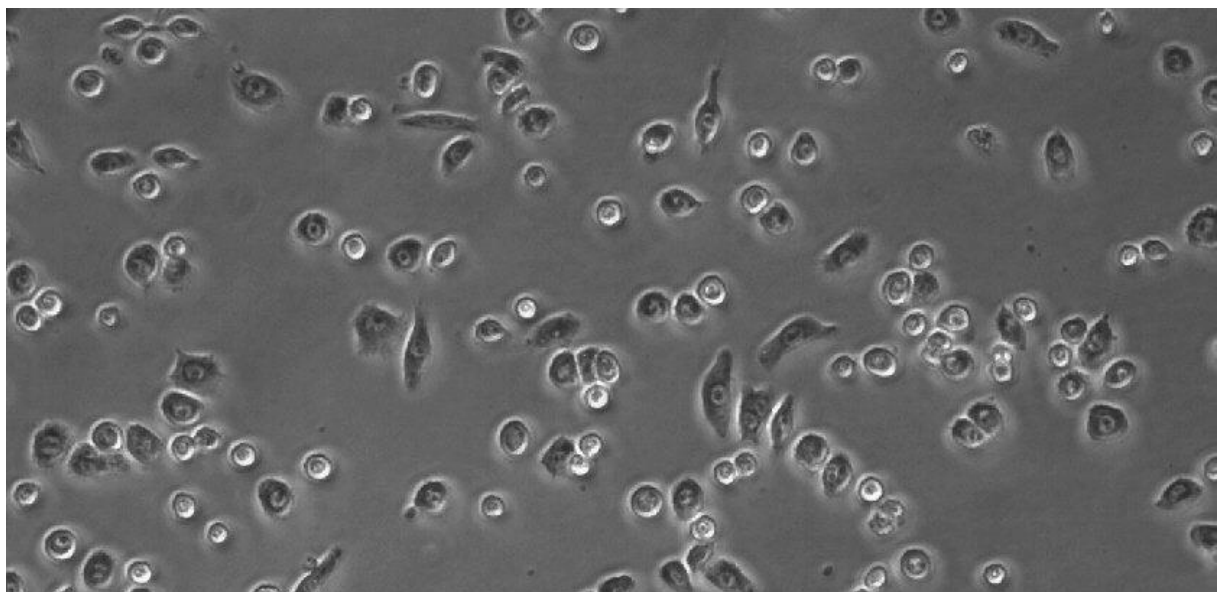


Fig. 5: RAW-264.7 in a μ -Slide 8 well, ibiTreat, after 24 hours incubation.

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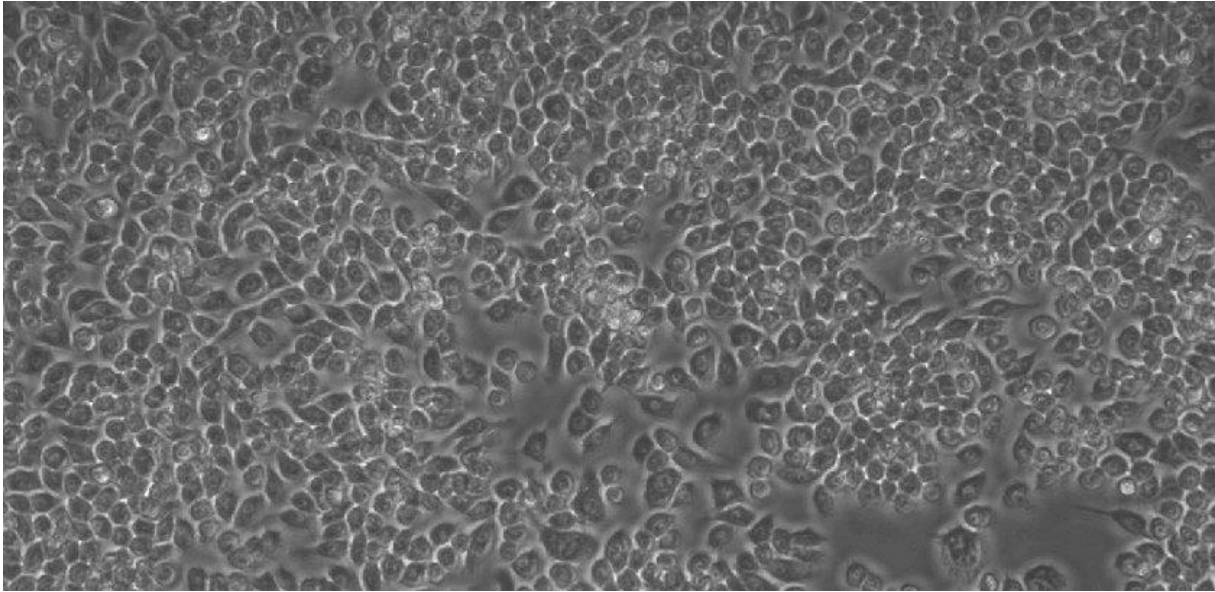


Fig. 6: RAW-264.7 in a μ -Slide 8 well, ibiTreat, after four days of cultivation.

For more detailed information about cell culture in the μ -Slide 8 well please also refer to the instructions on www.ibidi.com.

For an optimal homogeneous cell distribution we recommend using a channel slide like μ -Slide VI^{0.4}. In μ -Slide 8 well, cell densities may vary from spot to spot over the whole surface of a well, depending on the handling during cell seeding.

5. Immunofluorescence Staining

Fix and stain your cells with the same solutions as usual. An explicit protocol for immunofluorescence staining in channel slides is given in [Application Note 09 \(Immunofluorescence in \$\mu\$ -Slide VI^{0.4}\)](#).

Note: Take care with the fluid handling while staining in μ -Slides:

μ -Slide VI^{0.4}: For changing the fluids aspirate both reservoirs first without draining the channel. If you are working with a vacuum device, take care that you don't put the tip directly onto the channel's inlet. Flush the channel three times with 100 μ l of the new solution. Add the new solution from one side, let it flow through the channel into the other reservoir and aspirate it from the other side until both reservoirs are empty. Take care that the channel never runs dry!

μ -Slide 8 well: In the μ -Slide 8 well no special precautions are necessary. Exchange the fluids like in any other dish or well plate.

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In the following, an example is given for a cell nucleus and actin filament staining with the following material:

Cell nucleus: DAPI (Diamidino-phenylindol-dihydrochlorid), SIGMA, 32670

Actin filaments: Phalloidin, Alexa Fluor® 488 Conjugate, LONZA, PA-3010

1. Fix cells with 3.7% PFA in PBS (pH 7.4) for 10 minutes at room temperature.
2. Wash cells three times with PBS.
3. Incubate cells with Triton X 100 (0.1%) for 5 minutes.
4. Wash cells three times with PBS.
5. Incubate cells with 1% BSA in PBS for 20 minutes.
6. Wash cells three times with PBS.
7. Incubate cells with Phalloidin Alexa Fluor 488 at a concentration of 0.2 μM for 20 minutes.
8. Wash cells three times with PBS.
9. Incubate cells with DAPI (0.5 $\mu\text{g}/\text{ml}$) for 10 minutes.
10. Wash cells three times with PBS.
11. Aspirate the channel completely and refill it with ibidi Mounting Medium. The sample is now ready for observation on the microscope. It can be stored for some weeks kept cool and dark.

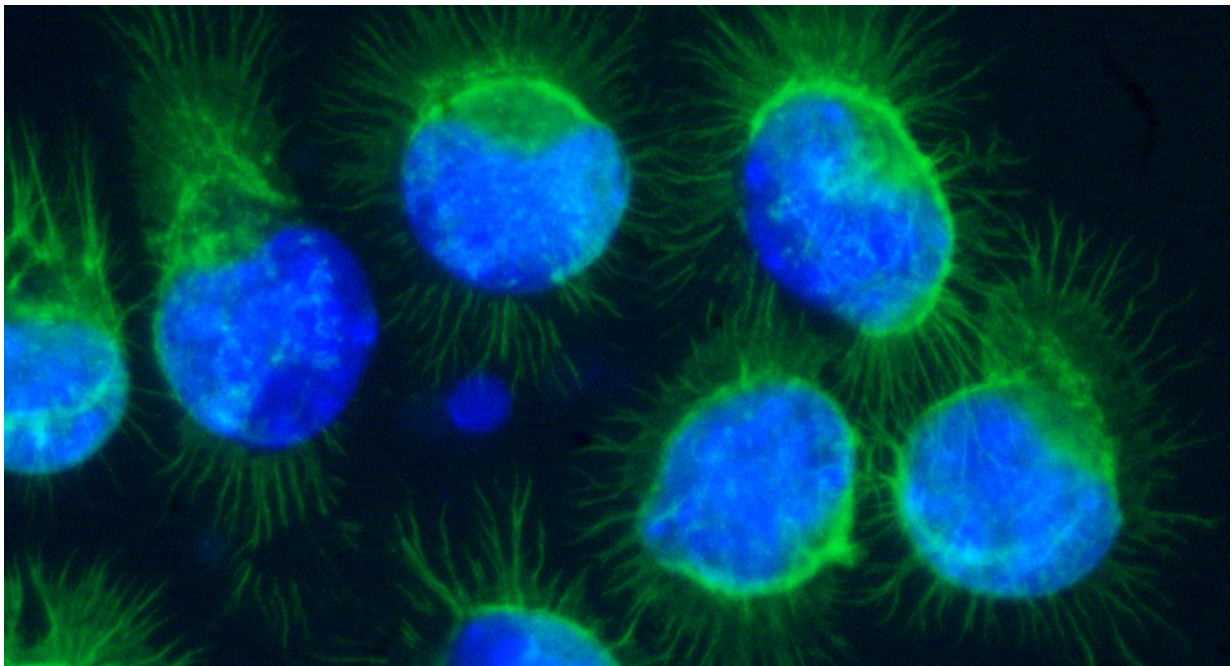


Fig. 7: RAW-264.7 in $\mu\text{-Slide VI}^{0.4}$ stained with DAPI (nucleus, blue) and phalloidin-AlexaFluor 488 (actin filaments, green).

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