emulate

Protocol for Emulate Organ-Chips:

Fixation and Immunofluorescence (IF) Staining

March 4, 2019

EP137 v1.0

Goals:	Key Steps:	Other Required Materials:
Fixing and IF staining of cells in Emulate Organ-Chips	• Fix cells in the chip • Permeabilizing and blocking for antibody staining • IF staining	• 4% paraformaldehyde • 10% saponin • BSA • PBS • Serum (animal species must match the species that the secondary antibodies were raised in) • Primary and secondary antibodies (depending on marker of interest to be stained) • DAPI for staining nuclei • P0.5–P1000 pipettes and tips

Note: This is the method we have developed for fixation and immunofluorescence staining for our Organ-Chips. We realize, however, that users may have their own fixation and staining processes that have been developed for specific cell types, antibodies, or antigens. If users would prefer to use other fixatives, permeabilizing solutions, or blocking buffers, they may do so while following the process outlined below. This protocol has been optimized for fixation and staining of the Emulate Liver-Chip for our specific staining protocols.

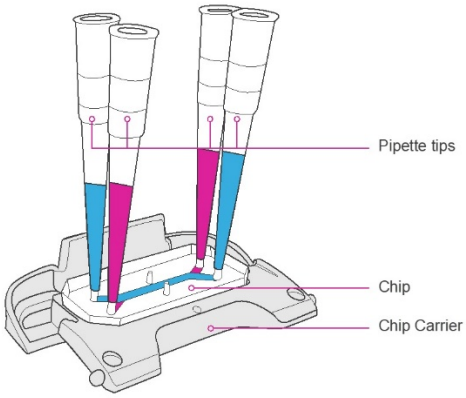
1. Method

Part I — Fixation of cells in Organ-Chips

1. Prepare the workspace of the chemical fume hood prior to beginning your work, ensuring that the space within the hood is organized, free from clutter, and the path of airflow is not blocked.

   Note: You will be using 4% paraformaldehyde (PFA in PBS) as part of this protocol. PFA is a hazardous chemical. Exposure risk to PFA can be greatly reduced by working in a chemical fume hood and using proper protective equipment for handling.

2. Ensure all chip carriers are labeled and identify the different conditions clearly. Detach chips from Pod™ modules, and organize them in petri dishes for handling.
3. Gently wash each channel with 200 µL PBS once.
4. Place 200 µL tips gently in the outlets of both channels. We recommend using filtered tips for this step. Be careful to not push the tips too hard against the bottom of the chip channel as this could seal off the outlet and prevent reagents from going through the channel and outlet.
5. Add 100 µL of 4% paraformaldehyde (PFA, in PBS, pH 7.4) to each channel from the inlet, leaving the tips inserted into the inlet as shown in the diagram below.



6. Incubate for 15 to 20 minutes at room temperature.
7. After incubation remove all four pipette tips and wash each channel with 200 µL PBS 3 times.

Note: Fixed chips can be stored at 4°C for up to one week in PBS. To ensure channels do not dry up during this period, it is recommended that PBS is added with 200 µL tips as described in steps 3 and 4 above. Then place the chips with tips in the ports inside plastic containers sealed with parafilm. We recommended using empty 200 µL tip boxes for storage. Ensure that the tips remain snug in the ports during transport and storage to avoid drying of the channels.

Part II — Permeabilization and Blocking

1. Prepare 1% saponin from 10% saponin stock in PBS.
2. Add 100 µL of this permeabilizing solution to both top and bottom channels, leaving pipette tips inserted in the ports as described in steps 3 and 4.
3. Incubate chips for 30 minutes at room temperature.
4. After incubation remove all pipette tips and wash each channel with 200 µL PBS 3 times.
5. Prepare blocking buffer by adding 10% serum (from the animal species the secondary antibodies were raised in) to the solution of 1% BSA in PBS.
6. Add 100 µL of blocking buffer to both top and bottom channels, leaving pipette tips inserted in the ports as described in steps 3 and 4.
7. Incubate overnight at 4°C. Alternatively, incubate for at least 2 hours at room temperature.
8. After incubation remove all pipette tips and wash each channel with 200 µL PBS 3 times.

Part III — Immunofluorescence Staining

1. Prepare primary antibody solution(s) for each channel by diluting the desired primary antibodies in 1% BSA-PBS. Note: Each channel can be stained with different primary antibodies. However, it is important to ensure unique host species for all primary antibodies to be used on a single chip to minimize cross-labeling and background noise between bottom and top channels.
2. The concentration of the antibodies will vary with each antibody used. This must be optimized prior to staining the chips.
3. After preparing the primary antibody solution(s), add 100 µL to the top and bottom channels, leaving pipette tips inserted in the inlet ports as described in steps 3 and 4. If you are only staining one channel, add PBS to the opposite channel.
4. Incubate chips overnight at 4°C.
5. After incubation remove all pipette tips and wash each channel with 200 µL PBS 3 times.
6. Prepare secondary antibody solution(s) for each channel by diluting the desired secondary antibodies in 1% BSA-PBS.
7. Add 100 µL of the secondary antibody solution to the top and bottom channels, leaving pipette tips inserted in the ports as described in steps 3 and 4. If you are staining just one channel, add PBS to the opposite channel.
8. Incubate chips for 2 hours at room temperature taking care to protect them from light.
9. After incubation, remove all pipette tips and wash each channel with 200 µL PBS 3 times. Optional Step(s):
    1. To stain nuclei, prepare a solution of DAPI diluted 1:1000 in PBS.
    2. If nuclei staining is desired, then add 100 µL of DAPI solution to the top and bottom channels each, leaving pipette tips inserted in the ports as described in steps 3 and 4. If you are staining just one channel, add PBS to the opposite channel.
    3. Incubate chips for 5 minutes at room temperature, taking care to protect the chips from light.
    4. After incubation, remove all pipette tips and wash each channel with 200 µL PBS 3 times.
10. The chips are now ready to image. Refer to Protocol EP126 Fluorescence Imaging for further direction.

Note: Stained chips can be stored in PBS for up to a week at 4°C. To ensure channels do not dry out during this period, it is recommended that PBS is added with 200 µL tips as described in steps 3 and 4 above, then place the chips with tips in the ports inside plastic containers sealed with parafilm and protected from light. We recommend using empty 200 µL tip boxes wrapped in aluminum foil for storage. Ensure that the tips remain snug in the ports during transport and storage to avoid drying of the channels.