OMEGA^{96} Microfluidic Microplate

User Guide

Contents

5524 Saint-Patrick, Office 460
Montreal, Quebec, Canada
H4E 1A8

CONTACT US AT INFO@ENUVIO.COM

v1.2

General Information

Unpacking

Thank you for purchasing eNUVIO's OMEGA^{96} microplates. Each plate has been carefully packaged under sterile conditions. To maintain its sterility, it is recommended to unpack the plate in an aseptic environment (e.g. in a biological safety cabinet). OMEGA^{96} plates are shipped "wet", that is their wells contain sterile filtered (0.1 micron) phosphate buffered saline (PBS; without divalents). The plates are ready-to-use for coating and/or seeding of cells.

Before Starting – IMPORTANT

OMEGA^{96} plates with the glass bases are fragile. To avoid cracking, plates should be handled with care, using only the outer black plastic plate frame when manipulating and moving the plate. Specifically, it is recommended to grasp the plate at the corners to avoid compressing the anti-evaporation reservoirs during manipulation. Compression of the reservoirs will not lead to damage of the frame; however, this can cause fluids that have been added to the reservoirs to overflow.

The wells of each plate have been sealed to prevent fluid loss during shipment, and the entire plate is packaged in a sterile bag. If the plate has been handled roughly during shipping, some of the shipping PBS can escape the sealer, and be found on the inside of the plate lid and/or the plate structure. **Leaks of this kind do not affect the sterility or functionality of the plate provided that (1) the bag has not been compromised, and (2) the device microchannels remain wet.**

Preparation for Use

It is recommended to prepare all reagents and tools required to carry out the protocol in its entirety prior to opening and removing the plate from its sealed packaging. The wells of the OMEGA^{96} plates have been sealed to prevent liquid loss during shipping. The plate sealer is removed by gently pulling on the white tab located at one side of the plate. **NOTE: remove the sealer using gentle and constant force to prevent damage to the upper structure of the plate.**

OMEGA^{96} plates are compatible with a variety of common downstream experimental procedures including:

a) Fixation and immunohistochemistry
b) Brightfield and fluorescence microscopy (e.g. widefield, confocal, TIRF, etc…)
c) Calcium imaging
d) RNA/Protein extraction and analysis (e.g. Western blotting)
e) Classical patch-clamp or optical electrophysiology

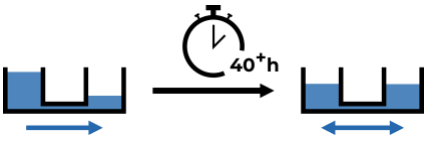
Surface Coating

To render the growth surface of each OMEGA^{96} well suitable for culturing cells, they must first be modified with a suitable coating. If plates have been purchased uncoated, these will need to be coated before seeding cells. The type of coating should be optimized for each culture/cell type that is being seeded in the plate. Some examples of common surface coating/modifying reagents include (not a complete list): poly-D/L-lysine, poly-D/L-ornithine, laminin, fibronectin and collagen. It is not uncommon for multiple coatings to be necessary for the successful seeding and growth of a culture. For example, iPSC-derived neuronal progenitor cells usually require coating with poly-ornithine (or poly-D-lysine) followed by a coating of laminin to be cultured successfully.

Flow Control and Asymmetrical Volume Loading

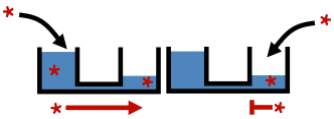
The OMEGA^{96} plate contains 48 individual chamber-pairs (for a total of 96 chambers), where each chamber-pair consists of 2 interconnected chambers that are joined by a series of 70 microfluidic channels. The direction of the flow of fluid across these high resistance microchannels can be controlled by adjusting the relative level of fluid in each of the chambers. It is the chamber fluid **level** that provides the force required to drive flow across the microchannels. Although there is a direct relationship between chamber fluid level (i.e. fluid height in the chamber) and fluid volume, it is the fluid level that primarily contributes to the force that will be applied across the microchannels. Consequently, it is differences in fluid levels that will provide the force required to drive fluid to flow from a chamber with a relatively higher fluid level towards a chamber with a relatively lower fluid level.

When two adjacent chambers joined by microchannels have identical dimensions, the relationship between chamber fluid level and volume is identical for each of the chambers. Therefore, directional flow across the joining microchannels can be easily determined by directly comparing each chamber's fluid **volume** (fluid will flow towards the chamber with a lower volume). However, in the case where two adjacent chambers do not have identical dimensions, the relationship between fluid level and volume will not be identical for the two chambers. Given that the volumes of adjacent chambers are known, it is possible to determine the level-to-volume ratio (level/volume) between the two chambers by simply calculating the volume quotient between the two chambers, and subsequently using this ratio to adjust chamber volumes accordingly. In this way, the directionality of the flow across the microchannels can be controlled. **All of the chambers in OMEGA^{96} plates have identical dimensions, and therefore chamber fluid volume can be used to simply adjust the directionality of the flow across the microchannels.**



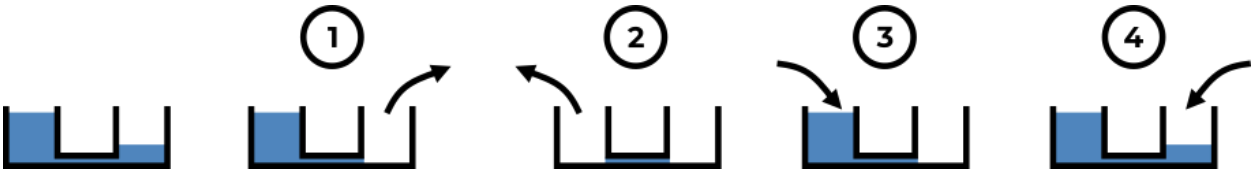
When adjacent chambers are loaded with different volumes of fluid for the purposes of driving a unidirectional flow across the adjoining microchannels, we refer to this as **"asymmetrical volume loading"** of the chambers. The unidirectional flow across the microchannels created by asymmetrically volume loading can serve to fluidically isolate the chamber with a relatively higher fluid level from any adjacent chambers containing relatively lower fluid levels. The flow will persist until the fluid levels (which supply the driving forces) in each of the chambers equalizes, at which point the directionality of flow will subside. Having reached an equilibrium, a slow bidirectional mixing of fluids will now occur between chambers. The duration of controlled unidirectional flow (e.g. for chamber isolation) depends on the **extent of the difference** in fluid levels between adjacent chambers. From the testing done on OMEGA devices, the unidirectional flow across the microchannels can be maintained for 40+ hours without adjusting chamber volumes. Accordingly, with regular verification and adjustment of the chamber fluid volumes, the unidirectional flow can be maintained perpetually.

When to Apply Asymmetrical Volume Loading



Asymmetrical volume loading of chambers is particularly useful when it is desirable to fluidically isolate one chamber from its adjacent, interconnected partner. Since the flow across the microchannels will be towards the chamber with the relatively lower fluid level, the chamber with higher relative fluid level **will not be** exposed to molecules that have been specifically added to the chamber with the lower fluid level. However, the chamber with lower fluid level **will be** exposed to molecules that have been specifically added to the chamber with the higher fluid level.

Chamber isolation can be maintained by simply maintaining the asymmetry of fluid levels between the chambers. However, care must be taken when exchanging media in each chamber to maintain the desired directionality of flow. Consequently, the order in which media is removed and replaced in each chamber needs to be considered when performing media exchanges. Media should be removed from the chamber with the lower level prior to removing the media from the chamber with the higher fluid level. Subsequently, media should be added to the chamber with the higher fluid level prior to adding the media in the chamber with the lower fluid level.



In addition to chamber isolation purposes, asymmetrical volume loading of chambers is useful when it is desirable to induce a flow through the microchannels. For example, this might be the case, when coating the microchannel surfaces or chemically fixing neuronal projections located within the microchannels. Also, asymmetrical volume loading is necessary to allow antibody access to epitopes located within the microchannels when performing immunohistochemical staining procedures.

Cell Seeding Density

The surface area of each chamber of the OMEGA^{96} plate is ~0.35 cm^2 (approximately equivalent to the area of a single chamber of a standard 96-well plate). Optimal plating density will depend largely on the nature and type of culture being plated in the device. It is therefore strongly recommended to conduct a series of optimization experiments to determine the ideal cell plating density. As a good starting point, seeding ~50 000 cells per chamber has been shown to yield good results using iPSC-derived neural progenitor cells (NPCs). For primary cultures, seeding density seems to vary by cell type, user, and lab. Some users have reported excellent results using a seeding density of as little as 30 000 cells per chamber, while others have had success seeding between 60 000 and 90 000 cells per chamber.

Anti-Evaporation Reservoirs

The osmotic pressure, pH and nutrient concentration of the culture media is critical for maintaining a healthy culture. This can be particularly problematic when having to maintain cultures for longer periods of time (weeks or months). Microplate wells are known to be particularly prone to evaporation, especially those located around the outer border of the plate. This can lead to the loss

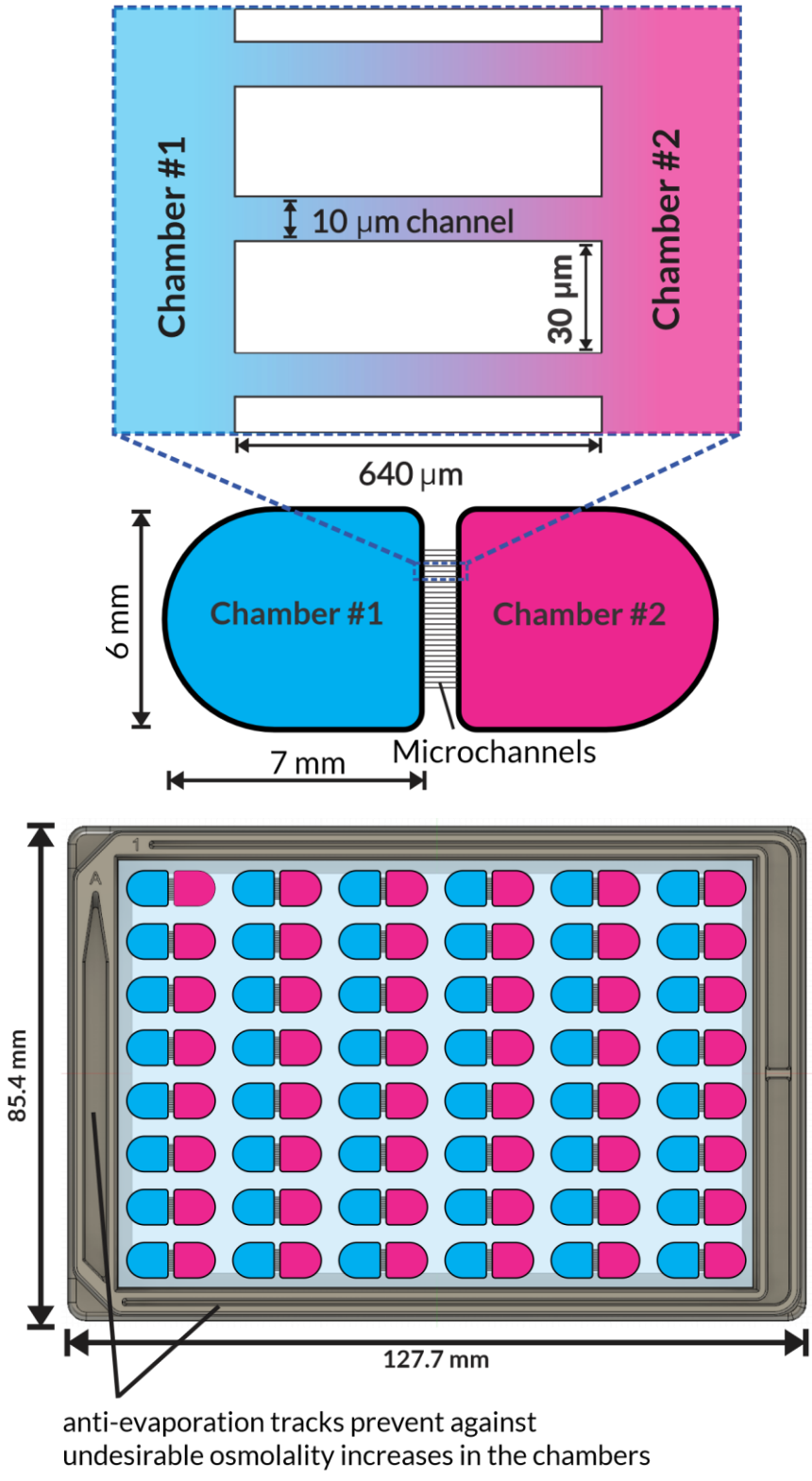
of the seeded culture (often to the surprise of the user) as the media gradually concentrates over time and can introduce unwanted variability in the data collected for the experiment. Unfortunately, these edge effects are so problematic that wells located along the outer edge of 96-well microplates are often left unused. To combat evaporation, all OMEGA^{96} plates have fluid reservoirs built into their black outer structure. When these are filled with liquid, they serve to reduce evaporation that occurs from the outermost chambers of the plate. In this way, these outer chambers can become available to be used in the experiment.

IMPORTANT: Although the culture evaporation minimizers do help to reduce evaporation rates during the incubation of cultures, they do not prevent evaporation. **Therefore, it is vital that the fluid level of each chamber of the device be verified and adjusted on a regular basis.** Verification frequency will depend on culture type, the number of times the culture is removed from the incubator, and on the environmental conditions (especially the humidity level) within the incubator. It is strongly recommended that the fluid level in both the anti-evaporation reservoirs and device chambers be verified every 2 days, exchanging culture media (e.g. 1/3 or 1/2 volume changes) and refilling them as needed.

Microscopy

Once cultures have been seeded, they can be examined over time using common microscopy techniques (e.g. brightfield or phase contrast). For example, OMEGA^{96} plates can be used for repeated live-cell imaging sessions using fluorescence markers, and/or fixed and immunolabeled with antibodies for immunohistochemical analysis. Immunochemistry processing, for example, can be performed by following the protocol provided in this user guide. The procedure can be used to label axonal processes located in the microchannels. Purposefully designed to conform to microplate dimensional standards, OMEGA^{96} plates are adapted to fit in most fluorescence microscope stages using universal microplate stage holders. Similarly, the pitch and relative position of each well conforms to 96-well standards, ensuring compatibility with automated machinery such as robotic liquid handlers, plate hotels/incubators, and high-content screening systems that are designed to function with standard microplate formats.

OMEGA^{96} Schematic



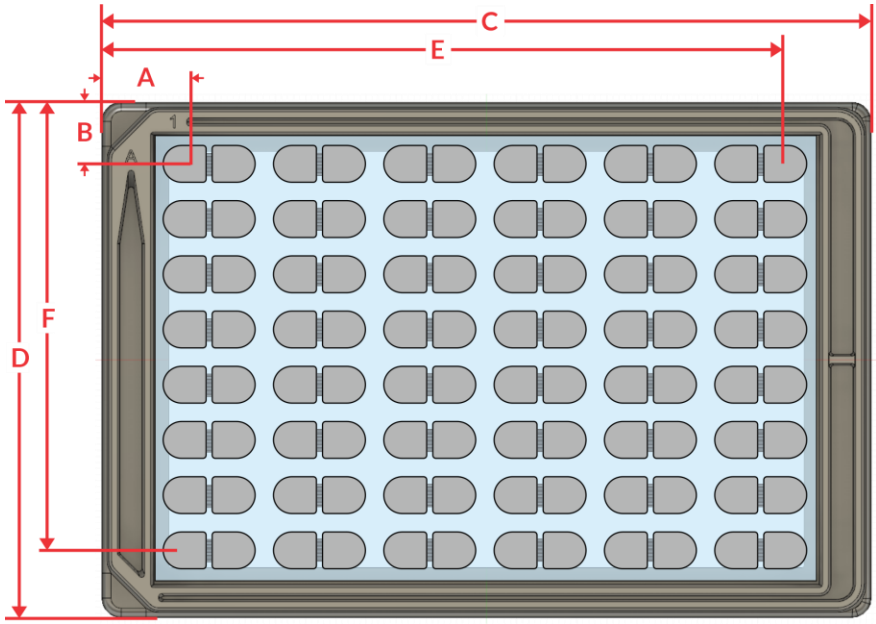
anti-evaporation tracks prevent against undesirable osmolality increases in the chambers

OMEGA^{96} Specifications

48 chamber pairs per microplate
Outer dimensions (w/ lid; *l* x *w* x *h*): 127.7 x 85.4 x 16.9 mm
Glass bottom thickness: 200 or 500 µm
Chamber dimensions: 6 x 7 mm
Chamber working volume: 100 - 325 µL
Max. chamber volume: 350 µL
Chamber surface area: ~0.35 cm^2
Number of microchannels per chamber-pair: 70
Microchannel length: ~640 µm
Microchannel width: 10 µm
Anti-evaporation reservoir (each): Recommended working volume: 1.6 mL (max. vol. 2 mL)

OMEGA^{96} Dimensions to Calibrate Automated Equipment

Please note it is strongly recommended to test and verify the calibration with your equipment.
(A) A1 to side offset: 15.5 mm
(B) A1 to top offset: 11.7 mm
(C) Length: 127.7 mm
(D) Width: 85.4 mm
(E) A12 to side offset: 112.5 mm
(F) H1 to top offset: 73 mm
(G) Height: 14.2 mm
(H) Glass bottom height from frame base: 1 mm
(I) Glass bottom thickness: 0.2 or 0.5 mm

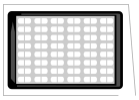


Protocol – Neuronal Cultures

General Information

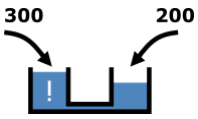
OMEGA^{96} microplates are shipped sterile. To maintain sterility, perform all relevant cell culture steps in a biological safety cabinet. Prepare all necessary reagents and consumables (media, PBS, tips, etc.) to complete the protocol prior to starting. If performing these steps manually, the use of a multichannel repeater pipette is recommended. **The following protocols are intended as a guideline only and the user is encouraged to modify this to best suit their experimental needs.**

General coating procedure (for uncoated OMEGA^{96} plates)
(skip to page 10 or 11 if using pre-coated plates)



i. Under aseptic conditions, remove the OMEGA^{96} from its plastic packaging. Remove the plate lid, and then the plate sealer. Apply a constant force to peel off the plate sealer by gently pulling on the white sealer tab.

ii. Remove the shipping PBS from each well using a vacuum apparatus. **Work efficiently to minimize the time the wells stay dry since the microchannels can quickly lose their hydrophilicity.**



iii. Add 300 µL of coating solution to one chamber of each chamber-pair.

iv. Add 200 µL of coating solution to the adjacent chamber of each chamber-pair.

v. Allow coating to proceed over 1 hour at room temperature. These are suggested incubation periods, coating times, temperature and concentration can vary according to experimental needs.

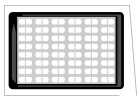
vi. Aspirate the coating solution, and wash by repeating **Steps iii. and iv.** with PBS or media. **Work efficiently to minimize the time chambers stay dry since the microchannels can quickly lose their hydrophilicity.**

vii. Leave the plate at room temperature for 30 minutes to allow for the exchange of coating solution from within the microchannels.

viii. If a second coating is required, repeat this process (**Step iii.**).

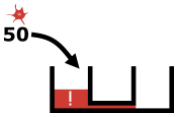
ix. Prior to seeding cells, remove all fluids from each chamber. Follow one of the seeding protocols below (beginning at **Step iii.**) for the intended experiment

Single neuronal cultures (compartmentalization purposes)



i. Under aseptic conditions, remove the OMEGA[96] from its plastic packaging. Remove the plate lid, and then the plate sealer. Apply a constant force to peel off the plate sealer by gently pulling on the white sealer tab.

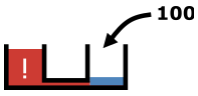
ii. Remove the shipping PBS from each well using a vacuum apparatus. **Work efficiently to minimize the time the wells stay dry since the microchannels can quickly lose their hydrophilicity.**



iii. Add the appropriate number of cells to 50 µL of media, and pipette this into one of the chambers.

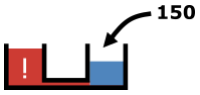
iv. Place the device in the incubator to allow the cells to settle and adhere to the surface (~10 minutes).

v. Once the cells have adhered, gently top up the seeded chamber by adding 250 µL of media (final volume 300 µL).

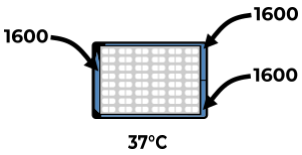


vi. Add 100 µL of media to the adjacent (empty) chamber.

vii. Incubate the device for at least 1 hour at 37°C.



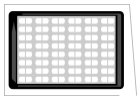
viii. Add 150 µL of media to the adjacent (empty) chamber (final volume 250 µL).



ix. Fill each anti-evaporation reservoir with 1.6 mL of water or PBS and return the plate to the incubator.

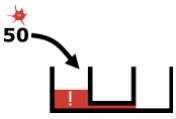
x. Over the course of incubation, monitor the fluid volumes of the culture chambers, exchanging the media as required by the culture. Ensure that each chamber contains the correct volume of media to maintain the desired experimental conditions (e.g. chamber isolation). Verify and refill the fluid in the evaporation minimizers as needed. Axonal outgrowth/co-cultures can require several days to weeks to fully establish

Establishing neuronal co-culture



i. Under aseptic conditions, remove the OMEGA[96] from its plastic packaging. Remove the plate lid, and then the plate sealer. Apply a constant force to peel off the plate sealer by gently pulling on the white sealer tab.

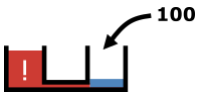
ii. Remove the shipping PBS from each well using a vacuum apparatus. **Work efficiently to minimize the time the wells stay dry since the microchannels can quickly lose their hydrophilicity.**



iii. Add the appropriate number of cells in 50 µL volume of media to one of the chamber pairs. Start by plating the neuronal culture (the culture destined for outgrowth).

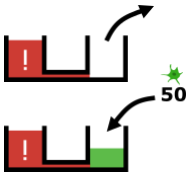
iv. Place the device in the incubator to allow the cells to settle and adhere to the surface (~10 minutes).

iii. Once the cells have adhered, gently top up the seeded chamber by adding 250 µL of media (final volume 300 µL).



iv. Add 100 µL of media to the adjacent (empty) chamber.

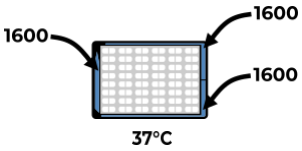
v. Incubate the device for at least 1 hour before seeding the second culture.



vi. When the second culture is ready to be seeded (could be days later), remove all media from the empty chamber, and then seed the second culture in 50 µL of media in the empty chamber.

vii. Place the device in the incubator to allow the cells to settle and adhere to the surface (~10 minutes).

viii. Gently add 150 µL – 250 µL of media to the second culture chamber and return the device to the incubator. The volume of media added here depends on whether asymmetrical volume loading is desired.



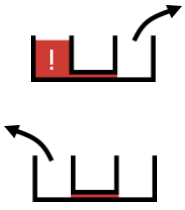
ix. Fill each anti-evaporation reservoir with 1.6 mL of water or PBS, and return the plate to the incubator

x. Over the course of incubation, monitor the fluid volumes of the culture chambers, exchanging the media as required by the culture. Ensure that each chamber contains the correct volume of media to maintain the desired experimental conditions (e.g. chamber isolation). Verify and refill the fluid in the evaporation minimizers as needed. Axonal outgrowth/co-cultures can require several days to weeks to fully establish

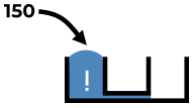
Protocol – Fixation and Immunohistochemistry

The following protocol is designed to fix and immunolabel culture within the chambers **as well as** processes located within the adjoining microchannels. Microchannel labelling is achieved by simply employing asymmetrical volume loading, in the same way that it may have been used for chamber isolation. Please note that using a volume ratio of at least 3:1 (e.g. 150:50 µL) across adjacent chambers has been shown to be optimal for immunolabelling epitopes contained **within** the microchannels. If immunolabeling within the microchannels is not required, there is no need to use asymmetrical volume loading and equal volumes can be used in both adjacent chambers.

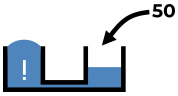
Fixation



1) Remove all solution from both chambers. If necessary, ensure chamber isolation (flow directionality) is maintained by removing solution from the **non-isolated chamber** (the chamber with the lower volume) before removing solution from the isolated chamber.

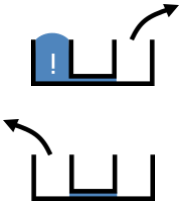


2) Carefully add at least 150 µL of **fixative** (e.g. 4% formaldehyde in PBS) to the isolated chamber. Note: this volume can be increased to as much as 350 µL, however this volume of solution may overfill the chamber and slightly "balloon out" of the top of the chamber.



3) Add 50 µL of fixative to the adjacent chamber.

4) Incubate the device at room temperature for 20 minutes.



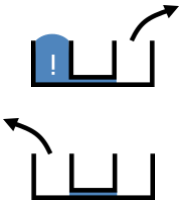
5) Remove fixative from both chambers. As in **Step 1**, begin removing solution from the non-isolated chamber first.

6) Wash the chambers by repeating **Steps 1 - 4** with **PBS**, observing the order in which chambers are emptied and refilled to maintain chamber isolation.

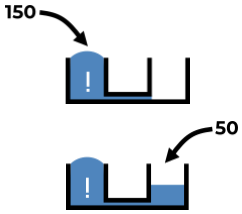
7) Repeat **Step 6** twice more, so that all chambers have been washed a total of three times.

Immunohistochemistry

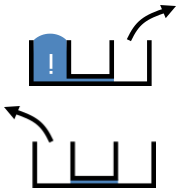
Blocking



8) Remove all solution from both chambers (maintain isolation where necessary).

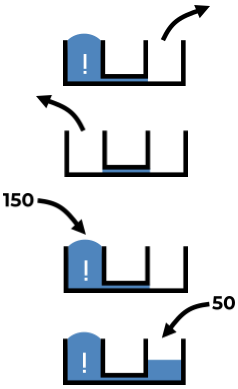


9) Repeat **Step 2 – 3** with **blocking solution** (e.g. 5 % normal serum, 0.2 % Triton X100, 0.05 % BSA), and incubate for at least 1 hour at room temperature (this can be also done overnight if desired).



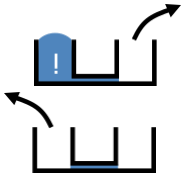
10) Remove blocking solution from both chambers. As in **Step 1**, begin removing solution from the non-isolated chamber first.

Primary Antibody



11) Repeat **Step 1 – 3** with **primary antibody solution** (dilution ratio(s) to be optimized).

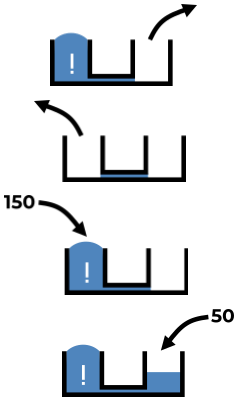
12) Incubate overnight at 4°C.



13) Remove primary antibody solution from both chambers. As in **Step 1**, begin removing solution from the non-isolated chamber first.

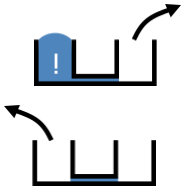
14) Wash the chambers three times with PBS as described in **Steps 6 - 7.**

Secondary Antibody



15) Repeat **Step 1 – 3** with **secondary antibody solution** (dilution ratio(s) to be optimized).

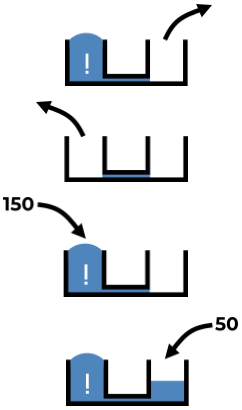
16) Incubate for 2 hours at room temperature.



17) Remove secondary antibody solution from both chambers. As in **Step 1**, begin removing solution from the non-isolated chamber first.

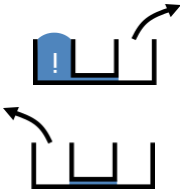
18) Wash the chambers three times with PBS as described in **Steps 6 - 7.**

Nuclear Counterstaining



19) Repeat **Step 1 – 3** with **nuclear counterstain solution** (e.g. Hoechst or DAPI; dilution ratio to be optimized).

20) Incubate for 5 minutes at room temperature.



21) Remove nuclear counterstain solution from both chambers. As in **Step 1**, begin removing solution from the non-isolated chamber first.

22) Add PBS solution to each chamber as described in **Steps 2 - 3.**