OMEGA$^{4\text{-}2mini}$ Starter Kit

User Guide

Contents

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v1.0

General Information

Unpacking

Thank you for purchasing eNUVIO's OMEGA$^{4\text{-}2\text{mini}}$ devices. All the items contained within the shipping vial have been carefully packaged under sterile conditions. To maintain sterility, it is recommended to unpack the contents of the shipping vial in an aseptic environment (e.g. in a biological safety cabinet). The shipping vial (and label) is completely autoclavable and can be repurposed (it is also recyclable).

The OMEGA$^{4\text{-}2\text{mini}}$ starter kit contains:

- 4 x OMEGA$^{4\text{-}2\text{mini}}$ devices (individually packaged)
- 4 x circular cell culture evaporation minimizers (reusable)
- 4 x 35 mm round culture dishes
- 1 x microscope stage adapter (reusable)

OMEGA$^{4\text{-}2\text{mini}}$ devices are packaged in sterile filtered (0.1 micron) phosphate buffered saline (PBS; without divalents) solution and are ready to use in cell culture. Each device is packaged sterile and are bonded to a 22 mm round #1.5 thickness glass coverslip.

Before Starting – IMPORTANT

Each device is double bagged to prevent loss of sterility during shipment. The inner-most bag containing the device is liquid filled, and this is placed in a sealed and sterile second bag. If devices have been handled roughly during shipping such that the inner bag may have been compromised, the sterile shipping PBS may leak and be trapped in the outer sealed bag. **Leaks of this kind will not affect the sterility or functionality of the device provided that (1) the outer bag has not been compromised, and (2) the device microchannels remain wet.**

Owing to its thinness, the glass coverslip that has been bonded to each device is fragile and must be handled with care. We take great care in packaging each device for shipment, however if the product is mishandled or handled roughly during shipment, the glass bottom may arrive cracked or broken. Cracks in the glass can easily be seen through the individual device plastic packaging, and therefore we strongly recommend that each device be inspected carefully **prior to opening the device's individual plastic packaging**. If any cracks within the glass coverslip are noticed, please send a photo of the damaged device in its unopened plastic sleeve including your order number to info@enuvio.com. We will be happy to quickly send you a replacement device. **Please note that we cannot provide replacements for broken devices if they have already been removed from their individual plastic packaging.**

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 device from its sealed packaging. It is crucial to prevent the microchannels from drying as this will cause the microchannels to lose their hydrophilic property (potentially within minutes). If the microchannels do dry, the device can be rejuvenated. This process involves thoroughly rinsing the device with deionized water, allowing it to dry completely, then oxidizing and sterilizing the device using a plasma or UV/ozone cleaner.

OMEGA$^{4\text{-}2mini}$ devices 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) Patch-clamp electrophysiology

Surface Coating

The OMEGA$^{4\text{-}2mini}$ devices are bonded to uncoated borosilicate glass. If required, steps should be taken to render the surface suitable for culturing the desired cell type. The type of coating and protocol for coating should be selected and optimized for each culture/cell type that is being plated on the device. Examples of common surface coating/modifying reagents include (not a complete list): poly-D/L-lysine, poly-D/L-ornithine, laminin, fibronectin and collagen.

Flow Control and Asymmetrical Volume Loading

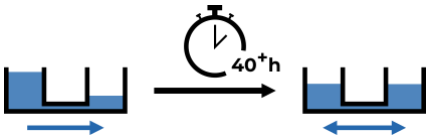
The OMEGA$^{4\text{-}2mini}$ device has 2 pairs of interconnected chambers, where each pair of chambers is joined via a series of microfluidic channels. The direction of the flow of fluid across these high resistance microchannels can be controlled by adjusting the relative level of fluid in each of 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.

The chambers in OMEGA$^{4\text{-}2mini}$ devices do not have identical dimensions. The surface area of the small chamber is 0.20 cm^2 whereas the large chamber surface area of 0.35 cm^2. Given equal depths of both chambers, a level-to-volume ratio of 1.75 should be implemented when calculating volume loading between small and large chambers. For example, loading the large chamber with 140 µL of fluid will result in a fluid level that is approximately equivalent to loading the small

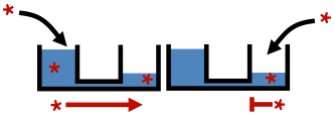
* may require the use of a 35 mm or slide microscope stage adapter

chamber with 80 µL (i.e. 80 µL x 1.75 = 140 µL). Since many scientists are familiar with liquid handlers that measure volume, the protocol provided in this user manual refers exclusively to chamber fluid volumes, and not levels, for clarity and ease of use.



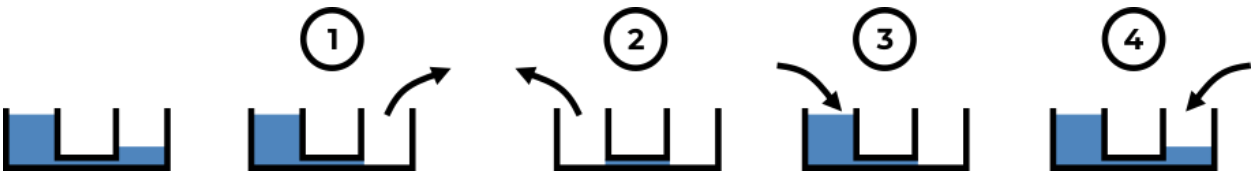
When adjacent chambers are loaded with different volumes of fluid for the purposes of driving a unidirectional flow across the adjoining microchannels, we refer to this as **"asymmetrical volume loading"** of the chambers. The unidirectional flow across the microchannels created by asymmetrically volume 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. 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 the larger chamber of the OMEGA$^{4\text{-}2mini}$ device is ~0.35 cm^2 (approximately equivalent to the area of a single chamber of a standard 96-well plate), whereas the smaller adjacent chambers have a surface area of ~0.20 cm^2. 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 in the large 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 large chamber, while others have had success seeding between 60 000 and 90 000 cells per large chamber.

Evaporation Minimizers

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). Due to their size and the way these devices are generally used, the small chambers are particularly prone to evaporation. **This is a common issue that results to unexplained poor culture health or complete loss of the seeded culture (often to the surprise of the user) as the media gradually concentrates over time.** For this reason, OMEGA kits come with cell culture evaporation minimizers that are filled with fluid to help reduce the evaporation rate from the OMEGA device chambers. These blue polydimethylsiloxane (PDMS) rings come packaged sterile and are designed to be reused (they can be sterilized using an autoclave or steam sterilizer for reuse). The inserts can be used as-is or can be rendered hydrophilic ("wettable") using a plasma or UV/ozone cleaner to facilitate fluid filling of the track.

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 evaporation minimizers 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.

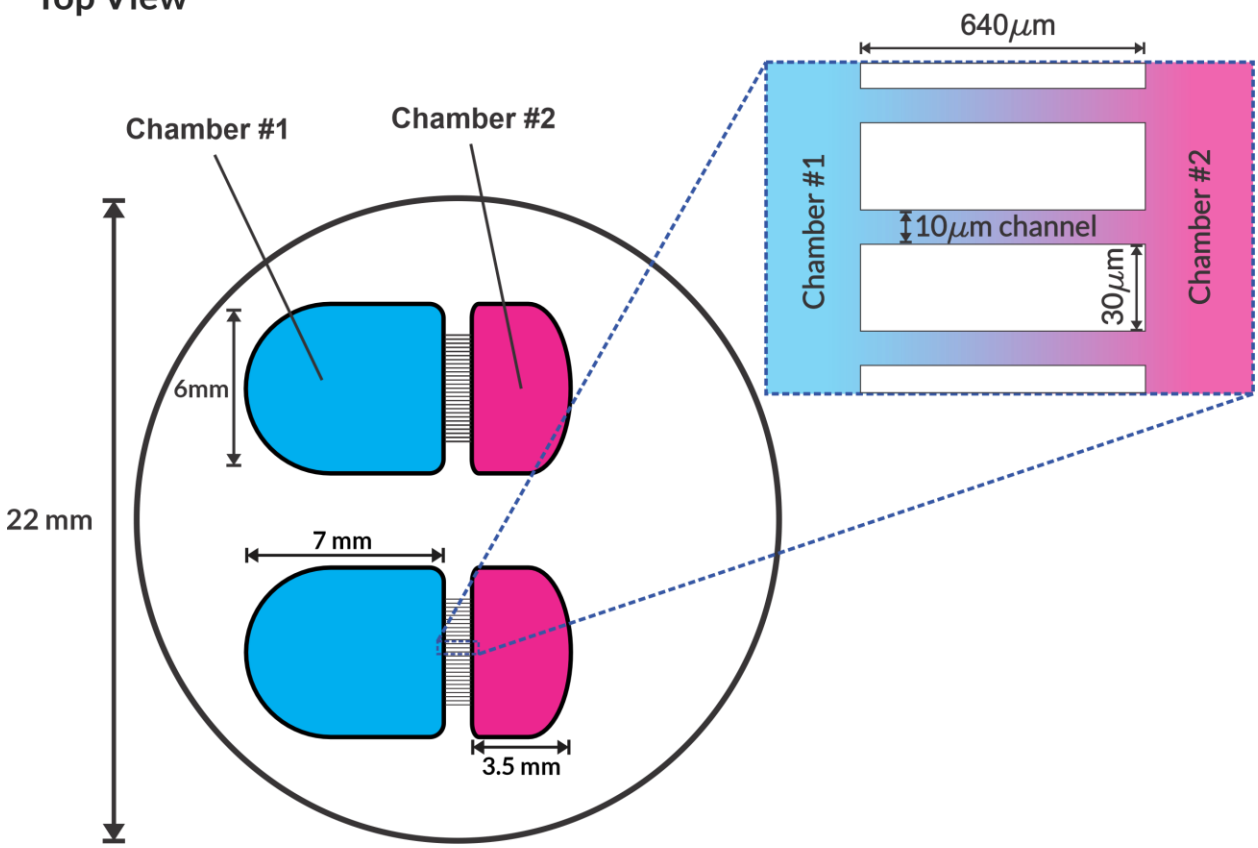
Microscopy

Once cultures have been seeded, they can be examined over time in their culture dish using common microscopy techniques (e.g. brightfield or phase contrast). The devices can also be setup for repeat live-cell imaging sessions using fluorescence markers, and/or fixed and immunolabeled

with antibodies for immunohistochemical analysis. The OMEGA device is **permanently bonded** to high-transmissive #1.5 thickness (0.16 mm - 0.19 mm) glass. T**he PDMS portion of the OMEGA device cannot be separated from bottom glass coverslip.** All processing for immunochemistry (for example) can be easily performed with the device fully intact (see protocol below) and has the added benefit of protecting the delicate axonal processes from detaching from the surface during the process. OMEGA devices are easily adapted to work with most fluorescence microscope stages using available stage holders (see protocol below).

OMEGA$^{4\text{-}2mini}$ Schematic

Top View



OMEGA$^{4\text{-}2mini}$ Specifications

Large chamber working volume: 40 – 150 µL
Small chamber working volume: 40 – 80 µL
Large chamber surface area: ~0.35 cm^2
Small chamber surface area: ~0.20 cm^2
Glass coverslip diameter: 22 mm
Glass coverslip thickness: 0.16 mm - 0.19 mm (#1.5)
Number of microchannels per interface: 70
Length of microchannels: ~640 µm

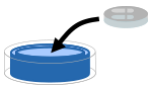
Protocol – Neuronal Cultures

Unpacking

1) Under aseptic conditions, place the blue cell culture evaporation minimizer into the bottom of the provided 35 mm culture vessel, ensuring the opening of the circular track reservoir is facing upwards.

2) Using a sterile blade or scissors, cut open the package of the OMEGA$^{4\text{-}2mini}$ device. This can be performed over a collection vessel to catch PBS that will drip during device removal.

3) Use a sterile flat-tipped tweezers or another suitable tool to carefully remove the device from its package. Take note of the device orientation. With the chamber openings facing up, gently dab the glass bottom coverslip with a wipe to remove residual PBS. Discard the packaging and the remaining packaging PBS.

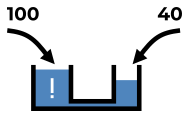


4) Place the device glass side down into the central opening of the blue evaporation minimizer.

5) Remove remaining PBS from each chamber using a vacuum apparatus or manual pipette that has been fit with a fine tip (10 µL or 200 µL pipette tips work well). **Work efficiently to minimize the time chambers stay dry since the microchannels can quickly lose their hydrophilicity.**

6) If steps are required to coat/prepare a glass surface for culturing cells, proceed immediately with these steps (see below). The working volume for the large chamber is between 40 - 150 µL, and between 40 – 80 µL for the small chamber. If coating microchannels, maintain an excess fluid volume (30 - 50 µL) in **only one** of the interconnected chambers (asymmetric volume loading). To help reduce evaporation from the chambers during incubation steps, add ~500 µL of sterile water or PBS to the circular track of the evaporation minimizer.

General coating procedure



1) Add 100 µL of coating solution to the large chamber.

2) Add 40 µL of coating solution to the small adjacent chamber.

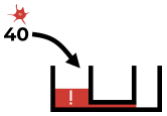
3) Place in the incubator for coating incubation period (generally 1 – 16 hours depending on the coating type/procedure; it is recommended to incubate the coating for at least 1 hour).

4) Remove coating solution. If required, the chambers can be washed with 70 - 100 µL PBS or media. **Work efficiently to minimize the time chambers stay dry since the microchannels can quickly lose their hydrophilicity.**

5) If a second coating is required, repeat this process with the second coating solution (**Step 1**)

6) Prior to seeding cells, remove all fluids from each chamber. Follow the correct protocol below for your intended experiment.

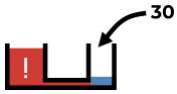
Single neuronal cultures seeded into the large chamber



i. Add the appropriate number of cells to 40 µL of media, and pipette this into the **large chamber**.

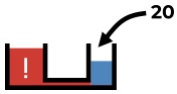
ii. Place the device in the incubator to allow the cells to settle and adhere to the surface (~10 minutes).

iii. Once the cells have adhered, gently top up the **large chamber** by adding 100 µL of media (final volume 140 µL).



iv. Add 30 µL media to the **small chamber** to prevent drying.

v. Incubate the device for 1 hour.

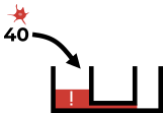


vi. Add 20 µL of media into the **small chamber** (final volume 50 µL).

vii. Return the device to the incubator. Monitor the fluid volumes of the culture chambers, exchanging the media as required by the culture (half- or third-volume media changes are recommended for neuronal cultures). 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.

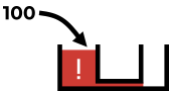
Establishing neuronal co-culture (seeding the large chamber first)

Note: If two different cell types will be co-cultured, start by plating the neuronal culture (the culture destined for outgrowth).

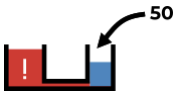


i. Add the appropriate number of cells to 40 µL of media, and pipette this into the **large chamber**.

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



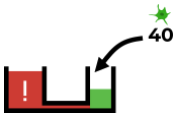
iii. Once the cells have adhered, gently top up the **large chamber** by adding 100 µL of media (final volume 140 µL).



iv. Add 50 µL media to the **small chamber** to prevent drying.

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

vi. When ready to seed the second culture (this could be days later if required), first remove all media from the **small chamber**.



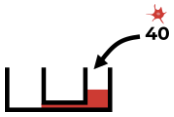
vii. Add the appropriate number of cells to 40 µL of media, and pipette this into the **small chamber**.

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

ix. Gently add 40 µL of media (80 µL total volume) to top up the **small chamber** and return the device to the incubator. **The volume of media in each chamber should be adjusted according to the desired experimental conditions (e.g., chamber isolation).**

x. 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.

Single neuronal culture seeded into the small chamber



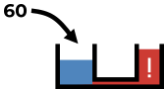
i. Add the appropriate number of cells to 40 µL of media, and pipette this into the **small chamber**.

ii. 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 **small chamber** by adding 40 µL of media (final volume 80 µL).

iv. Add 40 µL to the **large chamber** to prevent drying.

v. Incubate the device for 1 hour.

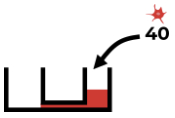


vi. Add 60 µL of media to the **large chamber** (final volume 100 µL).

vii. Return the device to the incubator. Monitor the fluid volumes of the culture chambers, exchanging the media as required by the culture (half- or third-volume media changes are recommended for neuronal cultures). 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.

Establishing neuronal co-culture (seeding the small chamber first)

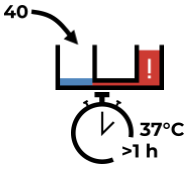
Note: If two different cell types will be co-cultured, start by plating the neuronal culture (the culture destined for outgrowth).



i. Add the appropriate number of cells to 40 µL of media, and pipette this into the **small chamber**.

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

iii. Once the cells have adhered, gently top up the **small chamber** by adding 40 µL of media (final volume 80 µL).



iv. Add 40 µL of media to the **large chamber** to prevent drying.

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

vi. When ready to seed the second culture (this could be days later if required), first remove all media from the **large chamber.**

vii. Add the appropriate number of cells to 40 µL of media, and pipette this into the **large chamber**.

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

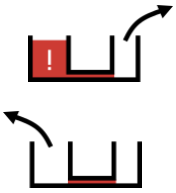
ix. Gently add 80 µL of media (120 µL total volume) to top up the **large chamber** and return the device to the incubator. **The volume of media in each chamber should be adjusted according to the desired experimental conditions (e.g., chamber isolation).**

x. 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.

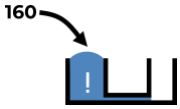
Protocol – Fixation and Immunohistochemistry

The following protocol is designed to fix and immunolabel culture within the chambers **including** 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. Maximizing the fluid level difference between adjacent chambers will increase microchannel fluid flow and will in turn maximize the immunolabelling of epitopes contained **within the microchannels**. In cases where immunolabeling within the microchannels is not required or desired, there is no need to use asymmetrical volume loading.

Fixation (if large chamber is isolated)



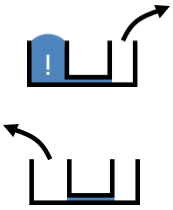
1) Remove all solution from both chambers. Ensure chamber isolation (flow directionality) is maintained by removing solution from the **non-isolated chamber** (the chamber with the lower fluid level; in this case the **small chamber**) before removing solution from the **isolated large chamber**.



2) Carefully add 160 µL of fixative (e.g. 4% formaldehyde in PBS) to the **large chamber** (isolated). Note that this volume of solution may overfill the chamber and slightly "balloon out" of the top of the respective chamber.

3) Add 40 µL of fixative to the **small chamber**.

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

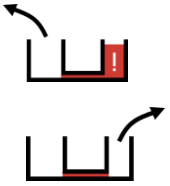


5) Remove fixative from both chambers. As in **Step 1**, begin by removing the solution from the **small chamber** first.

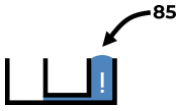
6) Wash the chambers by repeating **Steps 1 - 4** with **PBS**, observing the order in which chambers are emptied and refilled to maintain chamber isolation.

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

Fixation (if small chamber is isolated)



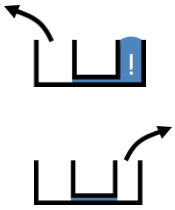
1) Remove all solution from both chambers. Ensure chamber isolation (flow directionality) is maintained by removing solution from the **non-isolated chamber** (the chamber with the lower fluid level; in this case the **large chamber**) before removing solution from the **isolated small chamber**.



2) Carefully add 85 µL of fixative (e.g. 4% formaldehyde in PBS) to the **small chamber** (isolated). Note that this volume of solution may overfill the chamber and slightly "balloon out" of the top of the respective chamber.

3) Add 40 µL of fixative to the **large chamber**.

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



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

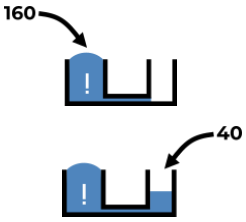
6) Wash the chambers by repeating **Steps 1 - 4** with **PBS**, observing the order in which chambers are emptied and refilled to maintain chamber isolation.

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

Immunohistochemistry

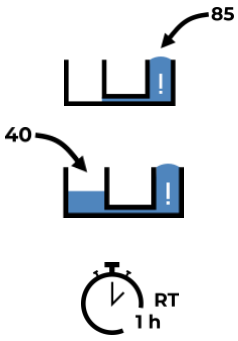
Blocking

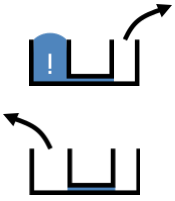
8) Remove all solution from both chambers (maintain isolation where necessary).



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).

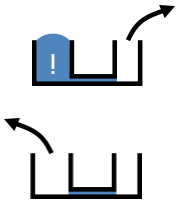
or



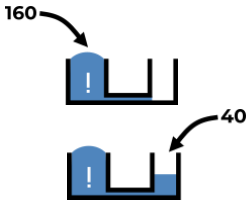


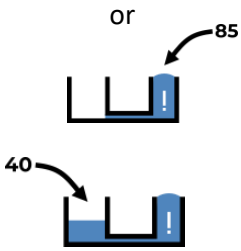
10) Remove blocking solution from both chambers. As in **Step 1**, begin by removing the solution from the non-isolated chamber first.

Primary Antibody

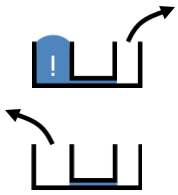


11) Repeat **Step 1 – 3** with **primary antibody solution** (dilution ratio(s) to be optimized).





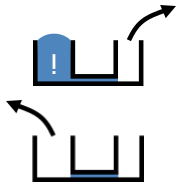
12) Incubate overnight at 4°C.



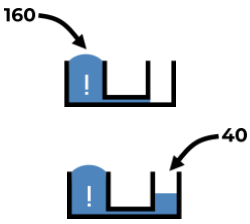
13) Remove primary antibody solution from both chambers. As in **Step 1**, begin by removing the solution from the non-isolated chamber first.

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

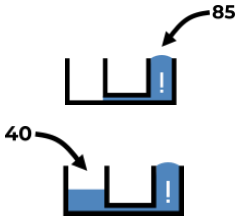
Secondary Antibody



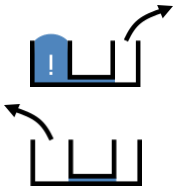
15) Repeat **Step 1 – 3** with **secondary antibody solution** (dilution ratio(s) to be optimized).



or



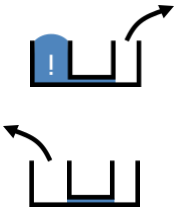
16) Incubate for 2 hours at room temperature.



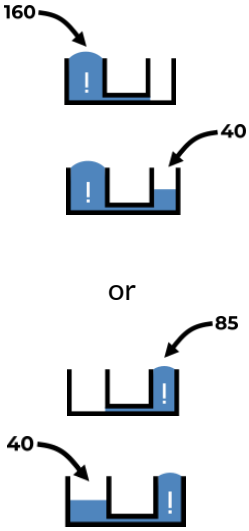
17) Remove secondary antibody solution from both chambers. As in **Step 1**, begin by removing the solution from the non-isolated chamber first.

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

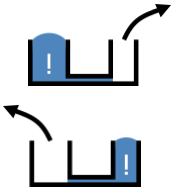
Nuclear Counterstaining



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.



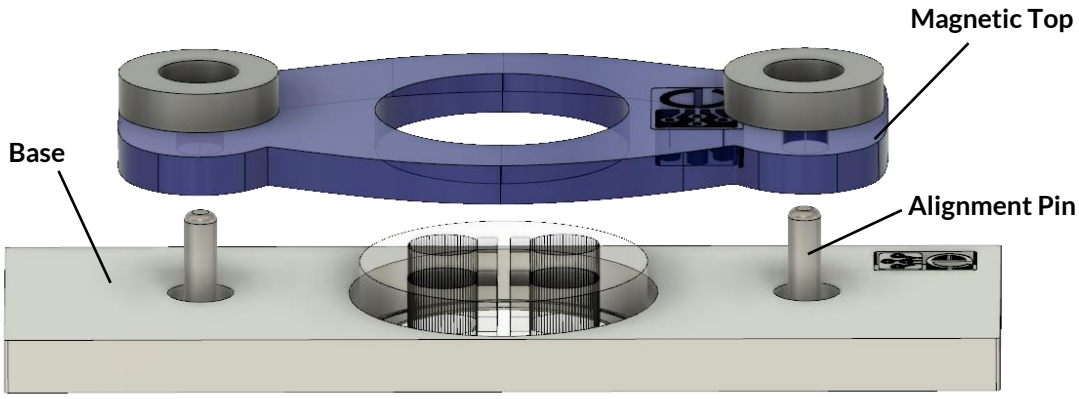
21) Remove nuclear counterstain solution from both chambers. As in **Step 1**, begin by removing the solution from the non-isolated chamber first.

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

Protocol - Microscopy

Slide-size Microscopy Adapter

This adapter is suitable for end-point imaging of OMEGA devices at low- and high-magnification imaging (5x – 100x). This low-profile adapter is compatible with oil immersion objectives since it provides adequate clearance for the relatively large size and shallow taper angles of many oil immersion objectives. The adapter properly stabilizes the OMEGA device on a level plane using magnets to “sandwich” the device between the magnetic top and the base. It has a 75 x 25 mm footprint which fits microscope stages which accommodate standard-sized glass slides. To use it, simply place the OMEGA device into the central opening of the base, then slide the magnetic top onto the alignment pins. Place the entire assembly into a universal standard glass slide accommodation commonly available on microscope stages.



Live-cell Microscopy Adapter (suitable for 4x – 20x magnification)

When performing repeated live-cell imaging, it is important to sterilize the microscopy adapter (autoclave or using 70% ethanol) and to carry out the assembly steps (see below) under aseptic conditions (i.e. in a biological safety cabinet). After establishing the culture, carefully remove the OMEGA device from the 35 mm plastic culture dish with tweezers or forceps and place it in the 35 mm round microscope stage adapter (see figure below). Position the device in the center of the adapter to expose the bottom glass, ensuring the device is level and flat. This assembly is designed to be used with microscope stage adapters that accommodate round 35 mm culture dishes.

To protect the culture during microscopy, use the lid from the 35 mm culture dish once the device has been placed in the microscopy adapter base. After imaging, return to the safety cabinet and replace the device into the original 35 mm culture dish (containing the evaporation minimizer). Check the volume levels of each chamber (adding or removing fluid if necessary), replace the lid, and continue to incubate the culture in the incubator.

Where additional stability is desired, a microscopy weight can be incorporated into the assembly (see figure below). The weight serves as an interface between the top of the OMEGA device and the bottom of the 35 mm dish lid, such that stage clips can be placed on top of the lid to stabilize the entire adapter assembly to a universal 35 mm stage holder. For live-cell applications, make sure to sterilize the weight using 70% ethanol, and assemble the chamber under aseptic conditions using the 35 mm culture dish lid to maintain sterility during imaging sessions (as described above).