AXIS™: *AX*on *I*nvestigation *S*ystem

For Catalog Numbers: AX15010, AX45005, AX45010, AX90010, and AX50010

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

upstate | CHEMICON | Linco

THE EXPERTISE OF UPSTATE®, CHEMICON® AND LINCO® IS NOW A PART OF MILLIPORE

USA & Canada Phone: +1(800) 437-7500 Fax: +1 (951) 676-9209
www.millipore.com

Introduction

The development of the vertebrate nervous system entails the generation and differentiation of neurons, establishment of signaling circuits, and the formation of numerous synapses. In particular, the formation of axons and dendrites during differentiation has been an area of intense interest. Although tremendous progress has been made in this area, neuronal outgrowth studies have been hampered by the absence of a solid, easy to use, neurite culturing platform which physically orients the cells during investigation. Traditional outgrowth experiments, conducted either by culturing neural stem cells, primary neurons, or tissue slices, typically result in random, overlapping neurite extensions. The ability to address questions surrounding the specific roles of axons, dendrites and somas in neuron differentiation and growth requires a faster, more specialized method that allows scientists to spatially isolate and study individual neurites and their function. To this end, Millipore now offers its most advanced tool for the isolation, visualization, and characterization of neurite outgrowth, the AXon Investigation System (AXIS™). AXIS™ is a unique, easy to use, slide-mounted microfluidic platform that allows culture, regulation, and directional differentiation of neuronal cells via a system of growth chambers and interconnected channels. AXIS devices physically isolate developing neurites from each other and their respective neural cell bodies. In addition, the use of hydrostatic pressure during analysis provides researchers with an opportunity for targeted exposure of neuronal parts to growth factors, drug compounds, or other reagents of biological interest. This ability to selectively control exposure of neuronal outgrowth makes AXIS a powerful platform for any researcher interested in understanding axons, dendrites, somas, and their roles in synaptic formation, neural cell development, differentiation, regeneration, degeneration, and trauma.

Application

Research studies that focus on the development of the nervous system typically seek to understand the cellular and molecular processes underlying the formation of neuronal circuits. These processes regulating neuronal differentiation can come from internal or external factors. Internal influences include the spatial and temporal expression of specific proteins such as transcription factors, cell surface receptors, or structural proteins. External influences include neurotransmitters, nutrients, toxins, and other stimuli which can mediate changes in cell function or activity. Therefore, an ability to easily address questions about neuronal differentiation and the specific roles played by internal and external factors in regard to axons, dendrites, and somas requires scientist to spatially isolate and analyze individual neurites and their function. To this end, Millipore now offers its most advanced tool for the isolation, visualization, and characterization of neurite outgrowth, the AXon Investigation System (AXIS).

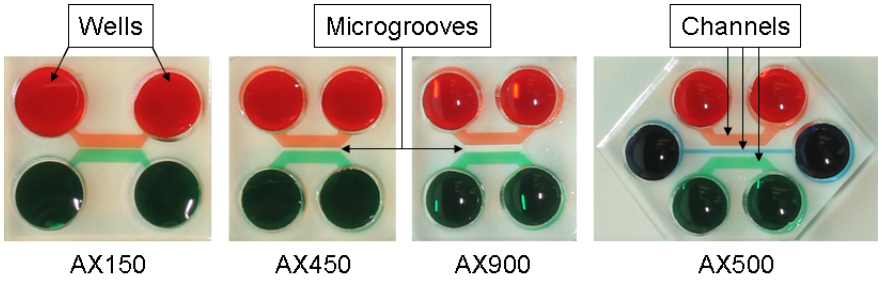


Figure 1: Pictures of the four different AXIS devices are shown. Examples of the three main features of these devices (wells, microgrooves, and channels) are identified. Please note that a chamber is composed of two wells with an interconnected channel and are depicted with red, green or blue coloring.

AXIS is a microfluidic device that enables researchers to easily isolate, observe, and test developing neurites. AXIS is offered in four different formats to provide test flexibility during investigation (Figure 1). The first three formats (AX150, AX450, and AX900) each have the same basic design consisting of four wells, two channels, and a set of microgrooves. Two of the wells are interconnected by a channel on each side of the device. The microgrooves are located in the area between the two channels and their length varies for each of the device formats; 150µm for AX150, 450µm for AX450, and 900µm for AX900 (Figure 2). Each device has approximately 120 microgrooves which are extremely small in size (approximately 5µm in height by 10µm in width). These microgroove dimensions were selected because they prevent cell bodies from flowing through while allowing neurite passage. The fourth AXIS device format that is offered contains 6 wells, 3 channels, and two separate sets of microgrooves (AX500). The AX500 is similar to the previous ones except a 500µm wide channel has been added in the middle and lies between the two sets of microgrooves which are each 500µm in length (Figure 1).

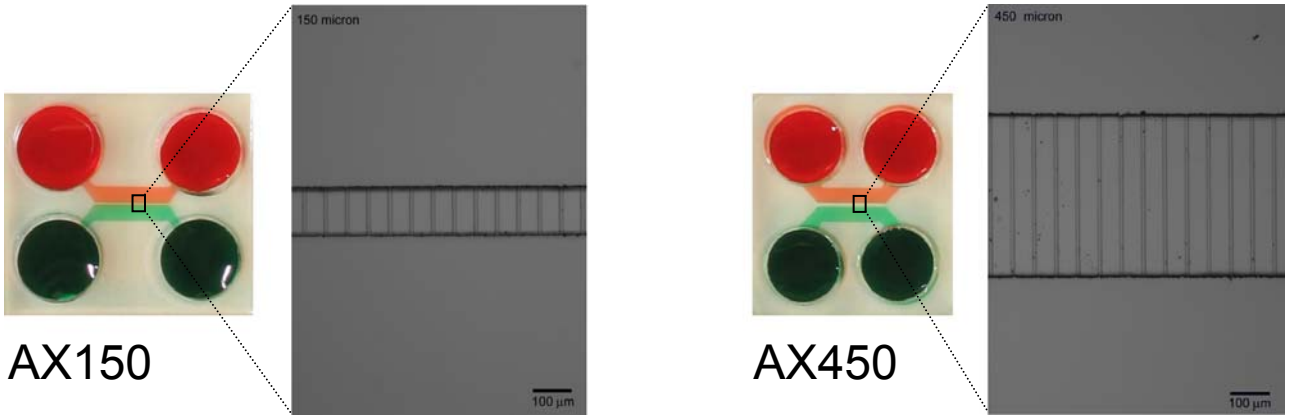


Figure 2: A higher magnification image of a portion of the microgroove area for two AXIS devices is shown. On the left is the AX150 device which has microgrooves that are 150µm in length. On the right is the AX450 device and its microgrooves are 450µm in length.

AXIS devices were carefully designed and offer numerous advantages over traditional testing methods. First, AXIS devices have been shown to effectively isolate cell bodies from differentiating neurites for a variety of cell types[1-5] and will likely work with most any neuronal cell type that can be successfully grown in tissue culture. Second, AXIS devices are fabricated from a polymer that is optically clear[6,7]. Thus, cells cultured in the device can be imaged with high resolution microscopy, including live cell imaging, confocal, and differential interference microscopy[1-5]. In addition, immunocytochemistry can also be performed within AXIS and a protocol to do so is provided below. Third, cultures grown using AXIS can be maintained for long periods of time (2-3 weeks of time routinely). This is made possible by the fact that the polymer that the device is made from is inert and non-toxic[6,7]. Finally, due to the microfluidic design of the AXIS devices they offer two additional benefits. The first is that they require only small amounts of test reagents to complete an experiment. Chemoattractants, toxins, recombinant proteins, radioactively labeled molecules, and other biological test reagents are often costly or in preciously small supply and their use in AXIS is minimized. The second benefit from the microfluidic design is that fluidic isolation is possible with it[2] therefore segregating chemicals to one side of the device. The basic design of the four well device creates two chambers (each composed of two wells and an interconnected channel) separated by a set of microgrooves (Figure 1). If the volume in both chambers is equivalent then there is equal pressure pushing from each side thereby limiting fluid flow to simple diffusion. However, if the volume in one chamber is greater than that in the other then hydrostatic pressure is created. This situation causes a slow but steady flow of liquid from the high volume side to the low volume side through the microgrooves. Fluidic isolation occurs when the hydrostatic pressure is selectively used to isolate the

solution on the low volume side. Since the fluid in the low volume chamber will not flow against the hydrostatic pressure coming from the high volume chamber, it remains almost exclusively on that side[2]. Therefore, a compound of interest can be exposed to specific cellular structures provided a hydrostatic gradient is established. Alternatively, a chemical gradient can also be established if the compound of interest is added to only one chamber. Over time, it will diffuse across the microgrooves and a gradient from high concentration to low will form. The microfluidic dynamics of the AXIS devices are key properties of the system and should be carefully considered during testing.

AXIS devices can facilitate neuronal experimentation in a variety of different ways and the test format can be relatively simple or highly complex. In the most basic assay, cells are differentiated within the AXIS device and cell morphology or protein expression is monitored by microscopy (Figure 3). Slight variations to this basic set up could include analyzing the affect a compound or reagent has on the cells during differentiation. Furthermore, it could also involve knocking out a protein and monitoring the affect it has on differentiation when exposed to a compound. More complex experiments using AXIS have recently been completed and include co-culturing of neurons with astrocytes following axonal extension[4]; axotomy assays in which axons are intentionally injured prior to analysis of expression patterns and structural changes during recovery[1,5]; and finally investigation into viral infectivity of neurons and the subsequent transport of viral particles within axons[3]. The options for using AXIS are extensive due to the variety of test formats available and the multitude of biological questions they can help address.

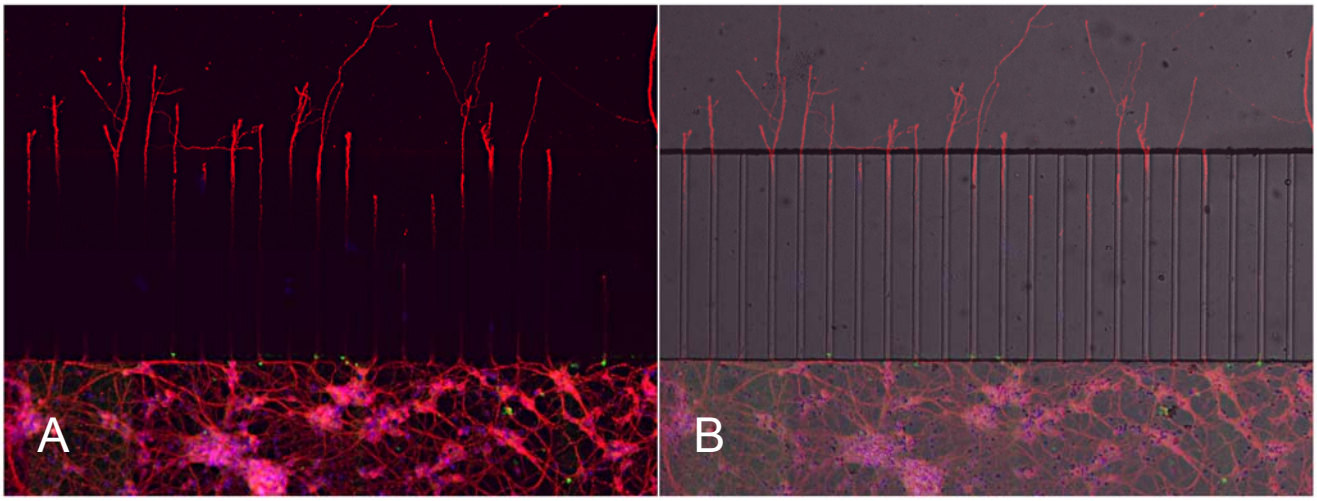


Figure 3: E18 rat hippocampal cells were loaded and cultured for five days in an AX450 device. Cells were fixed and triple staining was performed using DAPI (S7113 in blue), anti-Map2 (AB15452 in green), and anti-βIII Tubulin (MAB1637 in red) as shown in panel A. βIII Tubulin is an axonal marker, Map2 is a dendritic marker, and DAPI is a stain for cell nuclei. Panel B is the same image as in panel A except with on overlay of the white light image to verify the location of the channels and microgrooves. Note that the cells are contained in the lower channel where they were loaded and only the axons extend through the microgrooves and into the opposite channel.

In conclusion, the AXon Investigation System (AXIS) makes the study of neuronal development easier by enabling researchers as they isolate, visualize, and characterize the biology of neurites.

For Research Use Only; Not for use in diagnostic procedures

Product Offerings (Note: each kit is sold separately)

AX15010: Contains 10 AX150 devices
AX45005: Contains 5 AX450 devices.
AX45010: Contains 10 AX450 devices.
AX90010: Contains 10 AX900 devices.
AX50010: Contains 10 AX500 (6-well) devices.

Materials Not Supplied

1. Tissue culture instruments/supplies (37ºC incubator with CO_2, laminar flow/tissue culture hood, growth media, flasks, etc.)
2. Sterile 1X PBS (Millipore Cat No BSS-1005-B)
3. Sterile Milli-Q™ water (Millipore Cat No TMS-006-B)
4. Glass slides (25 X 75 mm) and coverslips (24 X 60 mm)
5. Human Collagen Type II (Millipore Cat No CC052), Laminin (Millipore Cat No CC095), Poly-D-Lysine(Millipore Cat No A-003-E), or some other cell attachment solution
6. 70% ethanol
7. 4% paraformaldehyde
8. Sterile plastic petri dishes (100 X 15 mm or larger)
9. Light Diagnostic Mounting Media (Millipore Cat No 5013)
10. DAPI (Millipore Cat No S7113)
11. Flat Jaw Forceps (like those used for holding western blot membranes)
12. Sonicator

Storage

The AXIS devices should be stored at room temperature in a clean dry area away from any chemicals or direct light. When stored properly the devices are stable for 6 months from date of receipt.

Assay Instructions

The AXIS system has been validated to work with a variety of neuronal cell types including primary cells and cancer cell lines. Listed below are detailed instructions about how to prepare the AXIS devices for use, maintaining neuronal cell cultures within them, and processing of them once an experiment has been completed. While these protocols have been extensively tested and validated to work, some optimization of testing may be necessary as not all cells have the same characteristics.

Please note that the AXIS devices provided are clean but not sterile. They must be sterilized prior to culturing cells in them to prevent any bacterial or fungal contamination. In addition, a glass substrate, typically a standard microscope slide, is required for testing but is not provided in the kit. The glass itself must also be cleaned, sterilized, and coated with cell attachment solution prior to use. Detailed protocols are given below for preparation of both AXIS devices and the glass substrate as well as adding the cells and subsequently culturing them during testing.

Glass Preparation

The coating of glass slides with cell attachment solution is a crucial step in the test process. Slides that are not properly coated will cause the neurons to clump and/or detach from the main channel during incubation. This can dramatically affect the number and quality of axons that are produced and thus able to traverse the microgrooves during differentiation. If a specific coating solution works for a particular cell type then that reagent should be utilized during initial testing using the AXIS system. AXIS devices can be mounted on either glass slides or coverslips during testing and it is user preference as to which to use.

1. Ensure that the glass slides and cover slips (24 X 60 mm) are free of contamination by sonicating them in Milli-Q water for 30-60 minutes.
2. Rinse the glass briefly with fresh Milli-Q water. If desired, the cleaned glass can be stored at this point in an enclosed container until ready for sterilization.
3. Thoroughly soak the glass in 70% ethanol for at least 5 minutes. Sterile plastic petri dishes work well for this incubation step and allow for the preparation of numerous slides simultaneously.
4. Transfer the petri dish containing the glass/ethanol to a laminar flow hood.

   Note: All subsequent steps should be performed in a tissue culture hood. From this point forward all testing should be done with sterile instruments and sterile reagents.
5. Aspirate the ethanol and rinse the glass with sterile Milli-Q water. Repeat the Milli-Q water wash then aspirate all remaining liquid.
6. Transfer a single clean and sterile glass slide into a new petri dish using sterile forceps (pre-soaked in 70% ethanol).
7. Pipette 500µL of sterile cell attachment solution onto the glass then gently place a clean, sterile coverslip onto it using forceps (alternatively two glass slides or two coverslips can be used depending upon user preference).

   Note: Higher concentrations of coating solution are generally required for effective cell attachment and are necessary due to the flow characteristics of the microfluidic devices. The following solutions and concentrations have been successfully implemented and are recommended starting points for testing: ***poly-D-Lysine at 0.5 mg/ml; laminin at 20 µg/ml; collagen at 50 µg/ml; and poly-L-ornithine at 0.5 mg/ml.***
8. Incubate for 2 hours in a tissue culture incubator or overnight at 4°C.

   Note: After the incubation, the glass slides are ready for use. If you intend to begin immediate axonal experimentation proceed to step 9. Otherwise transfer the petri dish containing the coated glass into a -20°C freezer for long term storage.
9. Remove the coverslip with forceps and gently rinse with sterile Milli-Q water.

   Note: Both the glass slide and the coverslip have now been coated. Be sure to keep track of which side of the glass was coated.
10. Repeat step 9 then dry the glass for 1 hour to overnight in a tissue culture hood.

The glass is now ready for immediate use. If desired, the coated glass can be stored at 4°C for up to 2 weeks prior to use.

AXIS Device Preparation

Please note that the AXIS devices come packaged with the microgroove imprinted side face down on a sheet of hard plastic within the sealed plastic pouch they are shipped in. It is important to always note the orientation of these devices during handling since the imprinted surface must be placed face down on the glass during cell culturing to function properly. If the device is accidentally placed upside down during testing it will not work. If at any point you are unsure which side of the device has the imprint simply examine the device carefully while reflecting light off of it. You should be able to see which side is printed with the channels and microgrooves. It is also important to note that during the handling of these devices be careful to not touch the area where the channels and microgrooves are located. Doing so could compromise the integrity of the devices and possibly cause issues such as leakage or broken microgrooves.

1. Open the pouch containing the AXIS devices in a tissue culture hood.
2. Remove a device using forceps and place it in a sterile petri dish with the printed side face up.

   Note: Multiple devices can be placed in a single petri dish depending upon how many are needed for testing. Any unused devices can be resealed in the bag using tape so as to prevent contamination with dust or debris.
3. Thoroughly coat the AXIS device(s) in the petri dish with 70% ethanol. Make sure that the alcohol completely covers all surfaces of the device.
4. Incubate in ethanol for 5 minutes.
5. Aspirate the ethanol and then let it air dry in the hood for 30-60 minutes.
6. The AXIS device is now ready for use. Ideally it should be used within 24 hours after sterilization.

Cell Preparation

AXIS kits have been functionally validated with numerous cell types including rat E18 cortical neurons, rat E18 hippocampal neurons, rat E14 dorsal root ganglion neurons, mouse E18 and P1 hippocampal neurons, mouse P2 cortical astrocytes, mouse E14 cortical neurons, N1E-115 neuroblastoma cells, and PC12 pheochromocytoma cells[1-5]. Given the diversity of cell types it has been successfully tested with, it would be expected that most any neuronal cell type that can be grown in culture would be compatible for use with AXIS. Furthermore, the offering of different microgroove formats should enable testing of any neurite producing cell.

The media and cell culture conditions standard for each specific cell type should also be employed during experimentation with AXIS devices. Cells to be utilized in testing can be prepared from actively growing cultures, frozen stocks, or freshly dissected tissues. Simply prepare the cells following standard protocols and then resuspend at a concentration of 3 to 5 million cells per ml in their normal growth/differentiation media. Although a high concentration of cells is recommended the total number needed per device is only about twenty thousand cells. The timing of when to begin preparing the cells is dependent upon how long the protocol for resuspension is and how long they can be incubated without side affects prior to use. Therefore, cells can be prepared prior to starting the standard protocol detailed below, during the 15 minute incubation at step 5, or once the device is nearly ready for use at step 8.

Researchers will have several options regarding cell culture conditions during testing with AXIS devices. Cells can be cultured from beginning to end in differentiation media. Alternatively, cells can be loaded into AXIS devices with growth media which is subsequently changed to differentiation media once the optimal cell density is obtained. Furthermore, chemotaxis agents can also be added

and they can be uniformly distributed or microfluidically isolated on either the axonal or cellular side of the device. All the various cell culture options will have to be considered and decided upon by the researcher prior to initiating testing.

Standard Protocol for Axonal Isolation

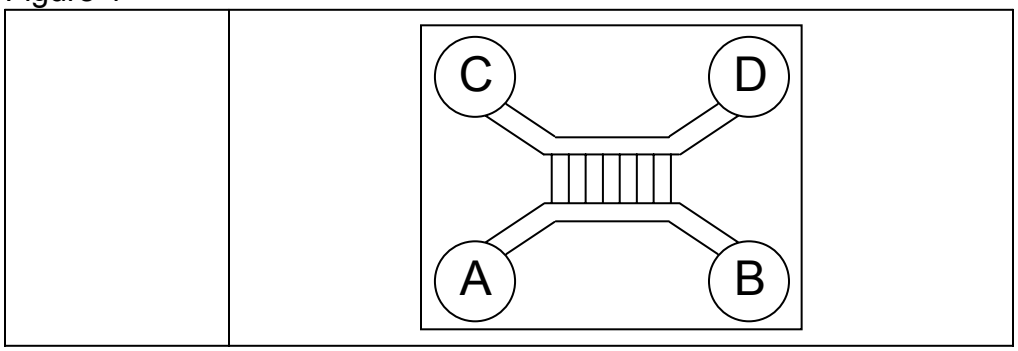
Once the glass has been coated with cell attachment solution and the AXIS device has been sterilized (following the protocols above) the system is now ready for set up and cell addition. All steps detailed below should be performed within a laminar flow hood using aseptic technique and sterile reagents.

1. Transfer a glass slide or coverslip (prepared above) to a sterile petri dish while ensuring that the coated side of the surface is face up.
2. Using forceps pick up one AXIS device and place it on the glass such that the imprinted side of the device is face down.

   Note: The channels and microgrooves form in the space between the glass and the AXIS device. The AXIS device is sticky by design, and will adhere to the glass once it comes in contact with it. If the device needs to be repositioned slightly pull it up and relocate it.
3. Use the forceps to apply firm but gentle pressure to numerous points all along the top of the device.

   Note: This will help seal the device to the glass and minimize any issues. This is not a permanent bond and care should be taken to minimize the disruption of it henceforth. The device, once placed on a slide, should look essentially as depicted below in Figure 4. ***Please take note of the orientation of the device as well as the well designations of A-D. All subsequent instructions will refer to the wells using these designations.***

Figure 4



4. Add 200µl of cell culture media to well A and allow the media to flow through the main channel into well B.

   Note: The media should flow through the main channel and into well B rather quickly. If it does not or if any air bubbles appear in the channel simply set your pipette to ~100µl and pipette up and down a couple times in well A with the pipet tip aimed at the entrance to the main channel. Be careful not to push the tip too hard under the AXIS device as it can become dislodged from the glass. If media still does not flow, verify that the AXIS device is oriented with the printed side down.
5. Once the media is through the channel, add 200µl of media to well B.
6. Incubate the device for ~15 minutes at room temperature.

7. Check the device on an inverted microscope to verify that the media has indeed passed through the microgrooves.

   Note: Be sure to cover the petri dish to keep it sterile during viewing. If the media does not flow to the opposite side through all microgrooves then let it incubate for an additional 15 minutes. If flow-through is still incomplete, pipette up and down (as in step 4 above) alternating between well A and B several times until expected changes are observed. Additional incubation may be required.

8. Add 100µl of media to well C, and allow the media to flow through the main channel into well D.

   Note: The media should flow into well D quickly. If it does not or if any air bubbles appear in the channel simply set your pipette to ~50µl and pipette up and down a couple times in well C with the pipette tip aimed at the entrance to the main channel.

9. Add 100µl of media to well D.

   Note: If you have not done so already, prepare the cells (as described in the "Cell Preparation" step above) for plating on the AXIS Axon Isolation Device. If your cells are ready, proceed to step 10.

10. Using a pipette or a vacuum, carefully aspirate the media from each of the four wells (A, B, C and D). Also remove the media from the main channel between wells A and B, but **DO NOT REMOVE THE MEDIA from the channel between wells C and D.**

    Note: To aid removal of media from the channel tilt the dish to drive the liquid out of one side.

11. Load 5µl of the cell suspension (3 to 5 million cells per ml) directly into the **channel** between wells A and B.

12. Place the AXIS device into a tissue culture incubator for ~10 minutes to allow cell attachment to the coated glass surface.

13. After 10 minutes check the cell count and adhesion level using a light microscope.

    Note: The optimal amount of cells to plate is user defined and dependent upon variables such as cell type, viability, media, extent of differentiation, etc. If an acceptable number of cells are present in the channel between wells A and B then proceed to step 14. If more cells are desired, add another 5µl of the cell mixture to the channel between wells A and B and incubate for an additional 10 minutes.

14. Add 100µl of media to wells A and B in rapid succession (preferably simultaneously). Repeat this step so that both wells A and B have a total of 200µl of media each.

    Note: Adding media to both wells in parallel will limit the amount of liquid flow through the channel minimizing cell displacement.

15. Check the cells using a microscope to ensure that:

    a. they are still attached in the channel,

    b. there are few air bubbles present,

    c. no cells are under the device, and

    d. no cells have flowed into or across the microgrooves.

    Note: Some media flow in the channel may be observed due to the microfluidic balancing of the volumes between wells A and B.

16. Add 100µl of media to well C, and allow the media to flow through the main channel into well D.

    Note: The media should flow into well D quickly. If it does not or if air bubbles appear in the channel pipette up and down a couple times in well C with the pipette tip aimed at the entrance to the main channel

17. Add 100µl of media to well D.

    Note: There will be twice as much media in wells A and B as there is in wells C and D. This setup will induce a hydrostatic flow of media from the cell attachment side toward the axonal differentiation side and should help drive any remaining air bubbles out of the microgrooves.

18. Place the petri dish with the AXIS device containing neurons into a tissue culture incubator to promote cell growth and/or differentiation overnight.

The cells and the media in the AXIS device should be monitored daily. The extent of cell differentiation and axonal extension into the microgrooves should be tracked microscopically. The volume and condition of the media must be observed and periodically changed. For most cell types it is ideal to remove and replace half of the media every three to four days. However, the frequency of media changes is dependent upon the cell type and the tissue culture incubator and will need to be determined empirically. Ideally, the number of media changes should be kept to a minimum to limit cell disruption. Media volumes should remain fairly constant but need to be monitored as excess evaporation can cause increases in salt concentration impacting cell health.

Plasma Bonding: An Alternative Protocol

Another method that can be utilized during testing with AXIS devices is plasma bonding. Exposure of AXIS devices and glass substrates to plasma gas causes surface activation which can be utilized to form a permanent seal between the two. Not surprisingly, there are advantages and disadvantages of plasma bonding versus traditional (i.e. non-plasma) bonding that a researcher will have to weigh as they decide which method to perform. Advantages of plasma bonding are essentially threefold. First, formation of an irreversible bond between the AXIS device and the glass substrate tends to minimize the risk of leakage. Second, after treatment a temporary conversion of the device from a hydrophobic state to a hydrophilic state occurs thus enabling the addition of protein coating solution. Finally, the last benefit is that plasma treatment will sterilize both the AXIS device and the glass substrate if performed properly. Plasma treatment does have a few potential disadvantages as well though. The first is that it requires specialized equipment (a plasma cleaner) to perform the bonding. Optimization of the plasma treatment may also be required to obtain a strong seal between the glass and the AXIS device and to ensure sterility. Second, the irreversible nature of the bond makes it impossible to remove the device for subsequent procedures such as microscopy. This may or may not be an issue depending upon the test objectives. As noted elsewhere the device does not have to be removed for viewing or immunocytochemistry but the clarity of the pictures are typically slightly better without it. Furthermore, the degree of staining of axons in the microgrooves is sometimes reduced as compared to that in the channels (likely due to decreased fluid flow). Since plasma bonded devices cannot be removed it may be difficult to get equivalent staining intensity on all cellular structures (see Figure 3 for evidence of this affect). Finally, confocal imaging with plasma bonding may be more challenging as the AXIS devices will have to be sealed to coverslips. Working with coverslips can be a challenge since they are more fragile and may require special stage setups on the microscope to hold them.

The protocol for plasma bonding of AXIS devices is provided on the Millipore website. If you are interested in finding out how to perform this procedure or reading more about it please go to www.millipore.com.

Microscopy

The ability to visualize and capture high resolution images of neurons that have differentiated through the microgrooves of the AXIS device is of great importance and is easily attainable. Facilitating this is the fact that the AXIS device is optically transparent; therefore, it is possible to monitor and capture cell differentiation in real time using live cell imaging. Furthermore, it is possible to carryout all steps of the immunostaining protocol with the device still in place. However, in some instances the axons in the microgroove area will not stain as strongly as the rest of the cell (likely due to reduced fluid flow), therefore if researchers require equivalent staining of their entire cell then it may be necessary to remove the device after fixing cells but prior to staining as noted below. Some optimization of the procedure may be necessary depending upon the cell type and the antibodies being utilized.

1. Carefully aspirate the media from the wells but do not remove it from the channels.
2. Wash the cells by adding 200µl of 1X PBS into wells A and B and 100µl into wells C and D.

   NOTE: ***Addition of these exact volumes into the respective wells should be maintained for all fluid additions in the subsequent steps unless noted otherwise. These unequal volumes will induce flow across the micro-grooves aiding testing.***
3. Incubate for 5 minutes at room temperature.
4. Carefully aspirate the 1X PBS from the wells but do not remove it from the channels. Repeat the 1X PBS wash two additional times.
5. Add 4% paraformaldehyde to the AXIS device (200µl into A & B and 100µl into C & D).
6. Incubate for 30 minutes at room temperature.
7. Wash with 1X PBS three times as detailed above.

   Note: If AXIS device removal is desired, it should be done so at this time. To minimize damage to the cells during device removal it is best to chill the slide for 5 minutes on ice prior to removing it.
8. Add blocking buffer to the wells (typically PBS with 1% BSA, 5% serum, and 0.2% Triton X-100).
9. Incubate for 1 hour at room temperature.
10. Aspirate the blocking buffer from the wells but do not remove it from the channels.
11. Add primary antibody diluted in blocking buffer and incubate at room temperature for 2 hours or overnight at 4°C.
12. Wash with 1X PBS three times for 5 minutes each as described in step 4.
13. Add secondary antibody diluted in blocking buffer and incubate at room temperature for 2 hours or overnight at 4°C.
14. Wash with 1X PBS three times for 5 minutes each as described in step 4 .
15. *Optional Step:* If desired add DAPI solution and incubate for 5-10 minutes.
16. Wash with 1X PBS two times as described in step 4, and fill all four wells with 200µl of 1X PBS.

The immunostained cells can be stored with 1X PBS at 4°C for a short period of time (~1 week) until they are analyzed using a microscope. Fluorescent images can be obtained with the AXIS device still attached to the glass using appropriate filters. Alternatively, the AXIS device can be removed at this point and the glass slide can be mounted using Light Diagnostic Mounting Media (Cat No 5013) prior to images being obtained.

References

1. Taylor, A., et al. 2005. A microfluidic culture platform for CNS axonal injury, regeneration, and transport. Nature Methods Vol 2 (8): 599-605.
2. Park, J., et al. 2006. Microfluidic culture platform for neuroscience research. Nat. Protocols 1: 2128-2136.
3. Liu, W., et al. 2008. A microfluidic chamber for analysis of neuron-to-cell spread and axonal transport of an alpha-herpesvirus. PLoS ONE Vol 3 (6): e2382 1-9.
4. Yang, Y., et al. 2009. Presynaptic regulation of astroglial excitatory neurotransmitter transporter GLT1. Neuron Vol 61: 880-894.
5. Taylor, A., et al. 2009. Axonal mRNA in uninjured and regenerating cortical mammalian axons. J of Neuroscience Vol 29 (15): 4697-4707.
6. Chun, Y.C. et al. 1992. Synthesis and characterization of poly(siloxane urethane)s. Polymer International Vol 27 (2): 177-85.
7. Shibayama, M. et al. 1991. Structure characterization of polyurethanes containing poly(dimethylsiloxane). Macromolecules Vol 24 (23):6254-62.

Warranty

Millipore Corporation ("Millipore") warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for a period of one year from shipment of the products. **MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications and descriptions of Millipore products appearing in Millipore's published catalogues and product literature may not be altered except by express written agreement signed by an officer of Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Millipore's sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies Millipore promptly of any such breach. If after exercising reasonable efforts, Millipore is unable to repair or replace the product or part, then Millipore shall refund to the Company all monies paid for such applicable Product. **MILLIPORE SHALL NOT BE LIABLE FOR CONSEQUENTIAL, INCIDENTAL, SPECIAL OR ANY OTHER DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGE SUSTAINED BY ANY COMPANY CUSTOMER FROM THE USE OF ITS PRODUCTS.**

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

For Research Use Only; Not for use in diagnostic procedures

** The AXIS product line is covered by US Patent 7,419,822. *