Neuro Probe, Inc.

Protocol for Using Neuro Probe Reusable Multiwell Chemotaxis Chambers

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Protocol for Using Neuro Probe Reusable Multiwell Chemotaxis Chambers

Neuro Probe reusable chambers are made of acrylic. Please refer to the cleaning and sterilizing instructions and other warranty information at the end of this protocol for proper care of this instrument.

This protocol refers to three different chambers, the AP48, AA12 and AA10. Exposed filter areas and well volumes are as follows:

Stock#	Number of Wells	Filter Area	Lower Well Volume	Upper Well Volume
AP48	48	8mm^2	25µL	50µL
AA12	12	18mm^2	150µL	100µL
AA10	10	50mm^2	400µL	285µL

Preparing the Bottom Chamber

Note: the following directions assume that you are working with polycarbonate filters. If you are working with cellulose nitrate filters, please contact Neuro Probe customer service to request staining protocols or find them on our website: www.neuroprobe.com.

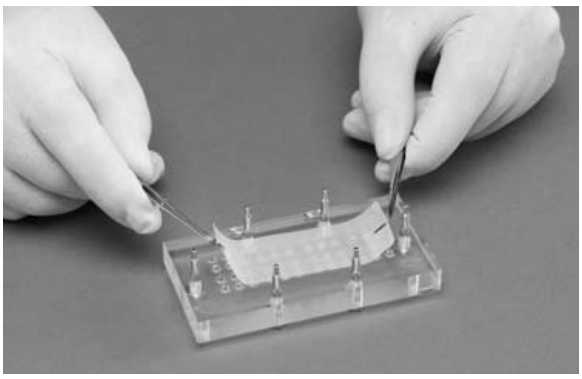

Figure 1

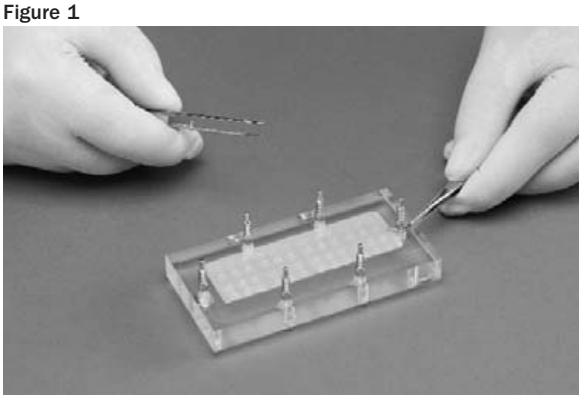

Figure 2

1. Adjust a variable volume micropipette with a 1mm tip so that the ejected liquid fills a bottom well. The volume should be such that a slight positive meniscus forms when the well is filled; this prevents air bubbles from being trapped when the filter is applied.
2. Orient the bottom plate on a flat surface so that the NP trademark is at the upper left. Warm chemoattractants or control reagents to about 37ºC and de-gas them by vortex or vacuum. Fill the bottom wells, completing the filling in no more than 5 minutes to prevent excessive evaporation.
3. Cut 1mm off the corner of a filter membrane and orient it with the cut corner at the upper left. Lift the filter by the ends with two forceps, hold it evenly over the filled wells, and lower it onto them, allowing the middle portion of the filter to make contact first *(see Fig. 1)*. The filter position can be adjusted at this point, if necessary, but note that too much movement will cause contamination between wells *(see Fig. 2)*.
4. Apply the silicone gasket with its cut corner at the upper left, then the top plate, aligning its trademark with the trademark on the bottom plate. Push the top plate down firmly and do not let go; this helps prevent air bubbles from being drawn in and trapped in the bottom wells. With your free hand apply and tighten the thumb nuts until finger-tight. Snug all the nuts, then tighten them alternately from side to side. Do not tighten one side first, as that may cause the top plate to rock and pull in bubbles. Do not use pliers or other tools to tighten them.
5. If you will be using an AP48 template (stock# P48TM) to help locate cell sites on filters, please refer to that protocol at this time.

Preparing and Adding Responding Cells

1. In the upper wells the concentration of cells in the media suspension should be adjusted so that the desired number of cells are contained in the appropriate volume. E.g., the exposed filter area for each well of an AP48 is 8mm^2, so a suspension of 8,000 cells in 50µL will yield 1,000 cells/mm^2, and 50,000 cells in 50mL will yeld approximately 6,000 cells/mm^2.

2. Pipette cell suspension into each upper well, adjusting the volume so that the filled wells have a slight positive meniscus. Hold the pipette at a steep angle so that the end of the pipette tip rests against the wall of the chamber just above the filter, and the side of the tip rests against the top rim of the well. Eject the fluid with a rapid motion to dislodge air in the bottom of the well.
3. Check for trapped bubbles in the upper wells. One easy way to do this is to look at the reflections of overhead lights in the meniscuses: a well with an abnormally large positive meniscus usually has a trapped air bubble. To remove any bubbles, suck the well completely dry with a suction line and disposable pipette tip, then refill it.
4. For most chemotaxis assays the filled chamber is incubated at 37°C in humidified air with 5% CO_2. Incubation times vary considerably depending on cell types and chemotactic factor(s). One good way to determine the optimum incubation time is to use 6-12 blind well chambers (e.g. catalog #BW100) set up as negative controls and placed simultaneously in the incubator. Remove one blind well after a set period (e.g. 30 minutes), and remove the rest sequentially, one every 5 minutes. Stain the filters and examine them to see how long it has taken for unstimulated cells to migrate through the polycarbonate filter. (Or, if you are using cellulosic filters, to migrate to a specified optimum depth.)

Staining Polycarbonate Filters

Implements described in the following procedures are available in an accessory pack (stock# P48AP), or they may be purchased separately.

1. Aspirate fluid from the top wells or empty them by shaking the chamber over a sink or container.
2. Remove the thumb nuts while holding down the top plate, and invert the entire chamber onto a paper towel. Grasp the four corners of the top plate (now on the bottom) and push down evenly so that it stays level as it drops to the table *(see Fig. 3)*. If the gasket should hang up on the post hardware, carefully push it down evenly onto the plate. Take care not to touch the filter, which should be stuck to the gasket. Immerse the remaining plate (with stud hardware in place) in cool distilled water.
3. The migrated cells are now facing up on the filter — this side of the filter is henceforth referred to as the "cell side." Lift up one end of the filter with forceps and catch 1mm of the edge in the wide plastic filter clamp *(see Fig. 4)*. Lift the filter and quickly attach the small SS clamp to the edge of the free end. Keeping the cell side up, wet the underside (non-migrated cell side) of the filter in a dish containing PBS *(see Fig. 5)*. Do not let the PBS wash over the cell side of the filter.
4. Holding the filter by the large clamp, with the small clamp attached to the other end and hanging free, wipe the cells off the non-migrated side of the filter by drawing the filter up over the wiper blade *(Fig. 6)*. The blade should first contact the filter just below the jaws of the wide clamp. Use only gentle pressure against the blade, and maintain an angle of about 30° from the vertical for the portion of the filter above the wiper. It is important to complete the wiping carefully and quickly so that the cells will not dry on the filter. Drying takes place in 10-20 seconds, and will prevent complete removal of the non-migrated cells.
5. Clean the wiper with a cotton swab, wet the underside of the filter again in PBS and repeat Step 4. Clean the wiper again, then wet the filter a third time in PBS and repeat Step 4.

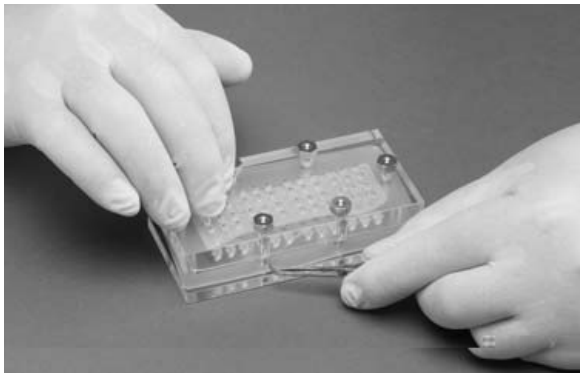
Figure 3

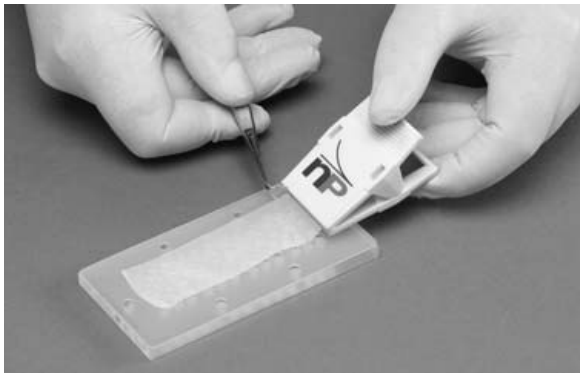
Figure 4


Figure 5

6. For granulocytes and monocytes, carefully immerse the filter in methanol (or other fixative recommended by your dye manufacturer), then place the filter cell side up on a disposable lint-free towel for air drying. Rinse all chamber components in cool distilled water. For other kinds of cells, consult the literature for staining techniques.
7. When the filter is dry, clamp the edge of one end with a large filter clamp, weight the other end with a small filter clamp and stain in Diff-Quik® or equivalent dye, according to the manufacturer's instructions. To avoid contaminating the chamber components with stain, it is convenient to have two sets of filter clamps, one for removing the filter from the gasket, and one for staining.
8. Place the wet filter cell side up on a 50 x 75 microscope slide to dry. When the filter is dry, center it on the slide and place a drop of immersion oil on it. Rub the oil over the filter with a smooth, blunt instrument to remove all bubbles and wrinkles. The filter is now ready for counting.

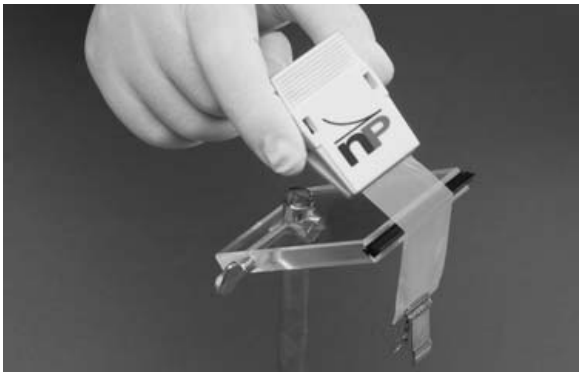

Figure 6

Cleaning After Each Use

This is a precision instrument; it should be handled and washed individually. Hard objects, including the hardware mounted in the bottom plate, should not be allowed to bang into the sealing surfaces of the chamber.

1. Soak components in distilled or deionized water without delay after each use to prevent proteins from drying on the instrument. The chamber may be reused immediately.
2. To clean after use, rinse all parts in running distilled or deionized water.
3. Remove residual water from each well in bottom chamber with a disposable pipette tip on a suction line, and let chamber components air dry at room temperature under a protective paper towel, or blow dry with *clean* compressed gas, e.g. nitrogen.

Periodic Cleaning

Proteins and other contaminants sometimes accumulate on the walls of the wells in the chamber and the gasket. This can happen quickly depending on the nature of the solutions used. Accumulation is immediate and severe if protein solutions are ever allowed to dry in the wells. Periodic soaking of all the chamber components in Terg-a-Zyme® or equivalent enzyme active detergent (available from most major laboratory suppliers) for 1-3 hours at 60°C, usually removes these residues. Follow with thorough rinsing under running distilled or deionized water and soaking in same overnight. Chambers can be cleaned this way as often as necessary; even weekly cleanings will not damage the chamber.

If continued contamination is indicated by the presence of erratic results, or by negative controls migrating faster than normal, the gasket is often the source of the problem. The gasket should be soaked in hot Terg-a-Zyme® at 100°C, and ultrasonically cleaned if possible. The gasket is very heat and scratch resistant, but very sensitive to solvents, which make it swell. A pipe cleaner may be used in the holes of the gasket (but not the chamber). If problems persist after this treatment, we recommend two courses of action. First, run some controls in blind well chambers parallel to the 48 well chamber. Since blind well chambers don't use gaskets, this will help determine if the gasket in your mulitwell is contaminated. Second, keep a spare gasket on hand and replace the old one.

Chamber Sterilization

Never autoclave chambers or expose them to solvents, including *alcohol*. Both can cause permanent damage to acrylic components.

If sterilization is required due to the presence of pathogens or for studies with long incubation times, acrylic and metal components may be sterilized in one of three ways:

1. gas sterilization with ethylene oxide
2. soaking for 30-60 minutes in 1 molar sodium hydroxide at 60°C, followed by thorough rinsing in sterile deionized water in a sterile environment
3. soaking in weak chlorine solution (1 T Clorox®/gallon distilled or deionized water) for 30-60 minutes, followed by soaking in sterile deionized water in a sterile environment. This will usually kill all bacteria; if other pathogens are present the strength and/or duration of the soak may need to be modified.

The *gasket only* may be autoclaved or boiled in deionized water. Avoid immersing the gasket in chlorine or sodium hydroxide solutions; it will absorb, and later bleed these solutions.

Failure to Adhere to these Warnings Immediately Voids the Warranty

- **NEVER** allow acrylic chamber components or silicone gasket to come in contact with solvents (e.g. ALCOHOL, acetone, carbon tetrachloride, etc.). **DO NOT** spray with solvents as preparation for work under a hood. Solvents create microscopic cracks in machined acrylic; when the instrument comes under normal stress (as in during assembly), serious fractures will develop at the points of greatest pressure.
- **NEVER** autoclave acrylic chambers; they will warp, deform or melt.
- **NEVER** place acrylic components in a drying oven or under UV light.
- **NEVER** immerse acrylic components of chamber in water hotter than 60°C. Ultrasonic cleaning of acrylic components is NOT recommended.
- **NEVER** allow solutions, especially ones containing proteins, to dry on chamber components.
- **NEVER** blow dry with compressed air containing oil or other contaminants.

References

Falk, Goodwin and Leonard, "A 48 Well Micro Chemotaxis Assembly for Rapid and Accurate Measurement of Leukocyte Migration," 1980, *Journal of Immunological Methods*, 33, 239-247.

Harvath, Falk and Leonard, "Rapid Quantification of Neutrophil Chemotaxis: Use of a Polyvinylpyrrolidone-free Polycarbonate Membrane in a Multiwell Assembly," 1980, *Journal of Immunological Methods*, 37, 39-45.

Richards and McCullough, "A Modified Microchamber Method for Chemotaxis and Chemokinesis," 1984, *Immunological Communications*, 13 (1), 49-62.

Harvath and Leonard, "Two Neutrophil Populations in Human Blood with Different Chemotactic Activities: Separation and Chemoattractant Binding," 1982, *Infection and Immunity*, 36 (2), 443-449.

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General Product Listing

Chemotaxis chambers

- Disposable – the ChemoTx® system
  - 96-well format
  - 384-well format NEW!
- Reusable chambers
  - 96-well chambers
    - MB-series microplate chambers
    - A-series 96-well chambers
  - Other multiwell chambers
    - AP48
    - AA12
    - AA10
  - Single well chambers
    - Blind wells
    - Boyden chamber
  - Specialty chambers
    - Chamber with filter retaining hardware (AC48)
    - 3-tiered chamber (A3BP48)
    - Cell orientation chamber (Z02)
- Accessories and filters

Protein Crystallography

- 96-well hanging drop crystallography system (CT300L2/5)

Cell settling & differential counting

- 8-well chamber
- Wicking agent
- Masked slides
- Centrifuge accessory pack