Cell seeding and use of Nanofiber Solutions multi-well plates*

Our culture plates are sterilized after packaging and may be used as direct replacements for tissue culture polystyrene in any traditional cell culture application. Nanofiber physically mimics the extracellular matrix found *in vivo* to provide a more realistic substrate to either culture or expand cells or to observe aspects of cell motility. These nanofibers are fully synthetic and are plasma treated to acquire whatever cell media components you normally employ. Before adding your cells we suggest rinsing once followed by a pre-incubation in the media + biological components of interest for at least 30 minutes and up to 24 hours at 37°C, aspirating off the media and finally adding your cells and media. If desired, specific protein coatings or gels (i.e., collagen or Matrigel) may be added to the as-received nanofibers using the same protocols as traditional culture dishes. Initial cell densities of 10^4-10^5 cells/cm^2 (the cell density can be more or less based on your cell type and experimental needs) are suggested. During longer-term experiments, exchange the media at normal rates.

Following proliferation/migration, cells+scaffold may be fixed for post-processing and/or stained for immunocytochemistry utilizing normal protocols. Bear in mind that these nanofibers are composed of biodegradable polycaprolactone which dissolves in organic solvents such as acetone or toluene. Fixing in 4% paraformaldehyde for 10-20 minutes, followed by a PBS rinse and permeabilization with cooled methanol for 5-10 minutes at –20 °C has worked well. Adherent cells can be trypsinized or lysed for gene or protein expression, microRNA analysis, etc. Many Nanofiber Solutions products are transparent to light to allow cells to be directly imaged using phase contrast or fluorescence microscopy.

*Suggested procedure, please adjust according to your experimental needs.