User Bulletin

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VioSpec 24-well 3D cell culture protocol
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The following is a general guideline for culturing of cell lines using VioSpec 3D cell culture system.

Note:
All cell culture must be undertaken in microbiological safety cabinet using aseptic technique to ensure sterility.

Preparing an aseptic environment:

1. Hood regulations
   (a) Close hood sash to proper position to maintain laminar air flow
   (b) Avoid cluttering
2. 2 Autoclaving
   (a) Pipette tips (or can be purchases pre-autoclaved, DNase/RNase free)
   (b) Glass 9" Pasteur pipettes
   (c) 70% ethanol. Be sure to spray all surface areas. All media, supplement and reagents must be sterile to prevent microbial growth in the cell culture. Some reagents and supplements will require filter sterilization if they are not provided sterile.
3. Preparation of cell growth medium before starting work, check the information given with the cell line to identify what media type, additives and recommendations should be used. Most cell lines can be grown using DMEM culture media or RPMI culture media with 10% Fetal Bovine Serum (FBS), 2 mM glutamine and antibiotics can be added if required. Check which culture media and culture supplements the cell line you are using requires before starting cultures. Culture media and supplements should always be sterile.

Subculturing from monolayer cell culture protocol:

1. Following the general monolayer cell culture protocol to the step of cell counting.
2. After counting the cell suspension, make appropriate dilutions of cells in desired cell culture media, it is recommended from $3x10^4$ to $3x10^6$ cells in 2ml of media.
3. Premix the cells and media and load 2ml cells/media suspension directly on a well of VioSpec 12-well plate.
4. Let the cells gently settle down to the white-color 3D matrix/scaffold, and place the culture plate into the cell culture incubator as general cell culture method, cells will settle and attach to the surface of the 3D matrix/scaffold, incubate for 3- 7 days, change the media in 1-2 days, and check the cells under a microscope
5. Under microscope, one should observe cells attach and grow on the 3D matrix/scaffold, the 3D matrix/scaffold contains a double layer matrix, cells should attach and grow on the bottom and upper layers, and in between two layers as well.
6. After 3-7 days, if cells grow as desired, using a pipetting tip to hook up the metal part of the 3D matrix/scaffold, and place cells-matrix/scaffold into a new well of a 12-well regular plate, cover with 2-3ml of new media, and incubate for another 3-7 days, during the period of incubation time, cells can be treated with desired treatment, such as drugs, hormones, antibodies, staining, etc.
7. Alternatively, after 3-7 days of growing, one can take up the cells-matrix/scaffold, and do desire experiments, such as, antibody labeling, staining, etc. Since, there are two layers of matrix, one can use a tweezer to separate upper and bottom layer, and do desire experiments, independently, such as, antibody labeling, staining, etc.
8. For harvesting cells from the 3D matrix/scaffold, the regular method can be applied, generally, trypsin can be used to remove adherent cells from 3D matrix/scaffold, cells are most commonly removed from the culture substrate by treatment with trypsin or trypsin/EDTA solutions.
9. To subculture cells onto a well of 3D matrix/scaffold. After cell harvesting, count the number of cells and then load a desire number of cells with 2-3ml of new media, and incubate for another 3-7 days.