How to use NanoCulture Plates

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Purpose

The purpose of this leaflet is to summarize SCIVAX Life Science's know-how to assist culturing 3D spheroids with NanoCulture Plate (NCP) technology.

Medium

1. Basal media containing 10%FBS is recommended
2. FBS should be heat-inactivated
3. Antibiotic, antifungal agent can be used
4. Additives such as growth factors are not recommended, due to promotion of monolayer cell growth
5. In case of monolayer growth using standard media, it is recommended that FBS be added between 1 to 20% for suitable culture conditions. Reducing FBS content is recommended when monolyer growth occurs.
6. NCM-R media was specifically developed for the culturing of well-formed spheroids. A similar effect with NCM-R can be obtained by adding 0.5 to 1% final concentration of Beckton Dickenson Matrigel™ to the media.
7. Please note that serum-free media may promote monolayer cell growth when used. Also, due to low surfactant action with serum-free media note that it will be difficult to remove micro-bubbles during pre-incubation.

Pre-incubation

8. Because NCP is made of a plastic with low wettability in combination with the nano-imprinted pattern, microbubbles can form on the well bottom which will likely result in monolayer growth. To prevent this, please refer to the following procedure for proper use.
9. This procedure will ensure the media will coat the entire well, particularly where the well wall meets the well bottom, and to remove micro-bubbles on the imprinted pattern.
10. Recommended pre-incubation procedure；
    I. Equilibrate media to room temperature.
    II. Add 50% of final media volume to the each well of the NCP plate, then centrifuge for 3-5 minutes at 1000xg to remove micro bubbles from the edge of the well bottom.
    Note: If you are not using centrifuge, please carefully remove micro bubbles by repeated, gentle pipetting, being mindful not to scratch the imprinted pattern on the well bottom.
    III. Incubate stationary for 15 -30 minutes, at room temperature or inside the CO_2 incubator.)

11. Recommended final seeding number for most cells for a 384 well plate is 3,000cells/well, 96-well plate is 10,000cells/well, and 60,000cells/well for 24well plate. Please consider seeding number condition by the cell types.
12. Seed an equivalent amount of cell suspension into pre-incubated NCP, and then keep the plate inside the tissue culture hood for 20-25 minutes to let the cells attach to NCP. If NCP plate place inside the CO_2 incubator before the cells attach to NCP, it may result in an uneven distribution of spheroids due to vibration and convection micro-currents while moving the plates.

    Note: after cell seeding, please avoid shaking the plate.
13. Spheroids can be observed by Day 3 after seeding, with a standard inverted microscope.
14. In case of changing media, carefully pipette 50% of media, and then replace with the same amount of media. This operation must be done as gently as possible being careful not to disrupt the spheroids. For long term cell culture, we recommend to change media once per week.
15. Unused wells can be used later, by covering the wells with a plate sealing tape (such as NUNC Cat#241205) and preventing contamination.