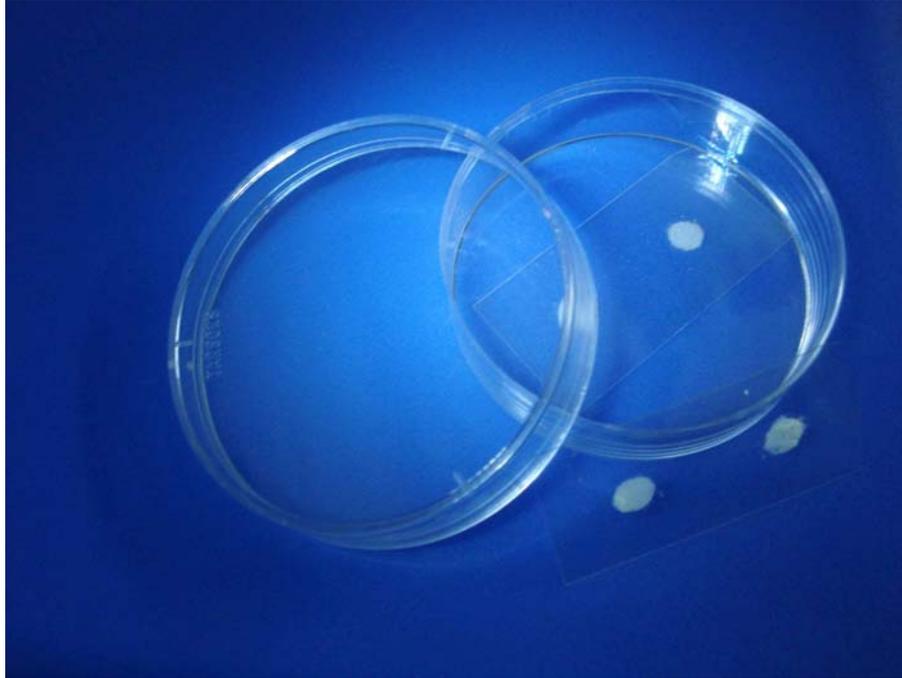


Confo-Cult®

3D Cell Culture for Confocal Microscopy*



Physiological relevance of three dimensional (3D) cell cultures has made it a fundamental research tool in cell biology¹. Hydrogels like matrigel and collagen are popular among cell biologists to create 3D tissue model due to their ability to mimic extracellular micro-environment^{2,3}. Porous scaffolds of biodegradable polymers on the other hand, are preferred for tissue engineering research for 3D cell cultures due to robust mechanical properties^{4,5}.

3D cultures are used in a broad range of cell biology research, including tumor biology, cell adhesion, cell migration, metastasis, angiogenesis and epithelial morphogenesis. It is expected to decrease laboratory animals in drug screening and toxicity assays¹.

Analysis of 3D cell culture requires delicate handling of 3D hydrogel from culture plates that may disturb

the cellular arrangements. 3D cell culture samples are highly scattering due to thickness (few hundred microns) which is a challenge for microscopic analysis.

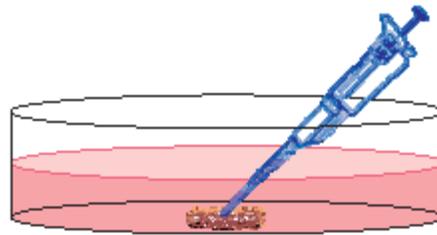
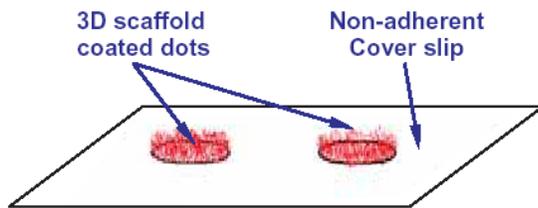
Researchers very often prefer to grow cells on cover slips as monolayers for confocal microscopy studies missing the advantages of 3D cell culture.

Innovations both in 3D cell culture formats and analysis techniques are essential to exploit the benefits of third dimension in the life sciences¹.

3D cell culture for advanced microscopy has been made easy and cost effective with Confo-Cult®. ECM Analog® coating of porous scaffold on typical cover slips makes it not only easier to perform 3D cultures but also simplifies confocal microscopic observations and high content analysis.

3D cell culture technology and products development is funded under Small Business Innovative Research Initiative scheme of Department of Biotechnology, Government of India

Confo-Cult®



Highlights

1. Confo-Cult® is designed for 3D cell culture for Confocal Microscopy.
2. Customizable Extra Cellular Matrix for 3D culture of specific cell types.
3. Customizable scaffold features.
4. Handling is identical to monolayer culture.
5. Cells do not grow on scaffold free area.
6. Just add cells, media, and incubate.
7. Multiple cell types can be co-cultured to create organ like features.

General Method

1. Confo-Cult® is supplied in a pre-sterilized ready to use Petri dishes.
2. Prepare cell suspension in complete medium.
3. Adjust the cell number as per the culture duration and cell multiplication for inoculation.
4. Add required amount of cell suspension in Confo-Cult® under sterile conditions.
5. Incubate in appropriate conditions, e.g. at 37°C, 5% CO₂ for attachment on the 3D dot for 24 hrs in an incubator.
6. After cell attachment on 3D dot of Confo-Cult® unattached cells are washed away twice with buffer and replenished with complete medium.
7. Alternatively cells in high concentration can be inoculated directly on the 3D dot in small volume (100 µL maximum). Cells are kept at 37°C, 5% CO₂ to attach for 2-3 hr and replenished with required amount of medium afterwards.
8. ECM Analog® coating can be pre-soaked if required with PBS/ medium for 24 hours before cell inoculation. Discard soaking medium before inoculation.
9. Routine observations can be made using typical microscopy. Normal bright field is likely to be convenient than phase contrast.
10. For confocal microscopy cover slip is retrieved and mounted as per the requirement

Precautions & Notes

1. Ensure that there is no residual protease enzyme in the cell suspension used for inoculation of Confo-Cult®. Protease enzyme will dissolve ECM Analog®.
2. Cell number needs to be adjusted depending upon duration of experiment, cell multiplication etc. For example, if cell have low multiplication potential or not required to multiply, cells may be inoculated in high concentration (0.1 million to a few million cells/ mL culture volume), i.e. typical saturation density of monolayer cultures.
3. Lid of Confo-Cult® dish is not shown in the illustrations for convenience.
4. Coated ECM Analog® may detach as small particles. These can be washed out during medium change.

References

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4. Lutoff MP, et al. Nature Biotech, 23, 2005, pp 47-55.
5. Mano JF, et al. J. R. Soc. Interface, 4, 2007 pp 999–1030.