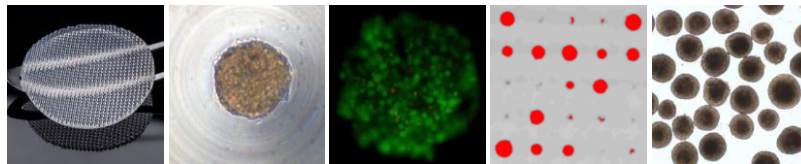# Application Note

Colony Forming Assay with 3D CoSeedis™

Introduction:

The colony forming assay (CFA), also known as clonogenic assay, describes a functional test on cell survival *in vitro*. It is based on the capability of a single cell to grow into a colony, which is defined to consist of at least 50 cells. The assay is particularly useful to determine the effect of chemical or radiation treatments on the self-regeneration of malignant cells, hence its widespread use in cancer research.[1,2]

CFA are cumbersome to perform following current protocols. Particularly the analysis of the assay is prone to cause substantial discrepancies depending on the person interpreting the results. Main reasons for those difficulties are the random distribution of colonies on different focal levels and coordinates using soft agar or 2D protocols, respectively.

3D CoSeedis™ now provides a novel protocol for CFA for adhesion-independent cells that fixes colonies in position and focal plane and allows (semi-) automatic binary analysis.

Procedure:

Seeding and cell growth
Cells are grown in the 3D CoSeedis™ matrix following the protocol for the *Organoid Standard System* using the 1 x 1 format.
The usually used cell density is 0.3 – 1 cells/microwell (cpm)
(for seeding, please also refer to the corresponding analysis tool on abc biopply's web site: https://biopply.com/products-applications/analysis-tool).
However, since the colony forming efficiency varies substantially, seeding density may be increased to 5 or more cpm depending on the cell type used. Furthermore, chemical or radiation treatments may also influence colony forming efficiency and

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[1] Franken NA *et al.*, Nat. Protoc. 2006; 1(5); 2315-9; 10.1038/nprot.2006.339
[2] Hoffman, Robert M. (1991), Journal of Clinical Laboratory Analysis; 5(2); 133-43; 10.1002/jcla.1860050211

consequently lead to a much higher seeding density per microwell. 3D CoSeedis™ tolerates a broad spectrum of colony forming efficiencies without sacrifying any precision in the analysis of the CFA.

Colonies are normally allowed to grow for 10 – 14 days.

Analysis

Analysis starts with the scanning of 3D CoSeedis™ matrices. Please follow the protocol *Volumetric Analysis* for step-by-step instructions (https://biopply.com/products-applications/protocols/).

Under paragraph 5, *Image Analysis*, bullet point 6, the cut-off to include any particular colony in the analysis is set to 20'000 – infinity. This ensures that non-colony forming cells are excluded from the binary analysis. However, the cut-off value may have to be adjusted depending on cell type, growth rate, etc.

The number of microwells showing colony growth is determined using abc biopply's analysis tool (https://biopply.com/products-applications/analysis-tool/) and the formula (=COUNT(area)).

Finally, the colony forming efficacy is calculated using Poisson statistics:

$$P(x = k) = \frac{\lambda^k}{k!} * e^{-\lambda}$$

With *P* as the probability to have an empty microwell, this leads to:

$$P(x = 0) = \frac{\lambda^0}{0!} * e^{-\lambda} = \frac{1}{1} * e^{-\lambda} = e^{-\lambda}$$

Where λ defines the average number of colony forming cells per microwell (cpm) and *k* equals the event that 0 cells within a microwell form a colony. *e* is the Euler's number.

Ultimately, the colony forming efficiency *E* can be calculated by:

$$E = \frac{\lambda}{cpm} = -\frac{\ln(P)}{cpm}$$

For a more detailed explanation including an example, please also refer to the white paper: **A binary approach to the colony forming assay: reliable and reproducible read-outs using 3D CoSeedis™** by *M. Leu et al.*

Additional information:

For additional information, please contact us under service@biopply.com or contact your local abc biopply partner.

Order information can be found at:
https://biopply.com/products-applications/order-3d-coseedis/