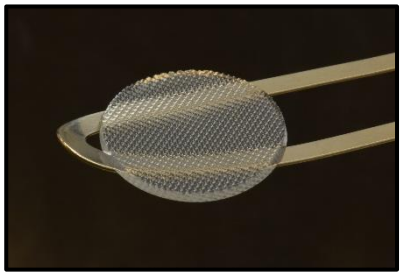
3D CoSeedis™

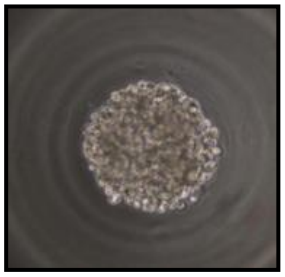
Easy Mass Production of Uniform and Homogenous Organoids for Predictive Disease Modelling

3D CoSeedis™ Standard Protocol

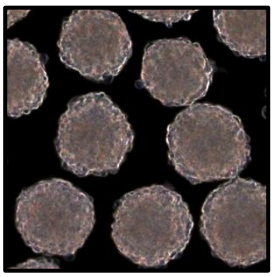
3D CoSeedis™ Chip680, compatible with 24-well plates



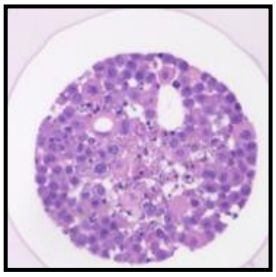
3D CoSeedis™ Chip680
for 24-well plates, ⌀ 15 mm



Spheroid in a microwell,
phase contrast



Harvested spheres



Paraffin section (H&E)

Order information

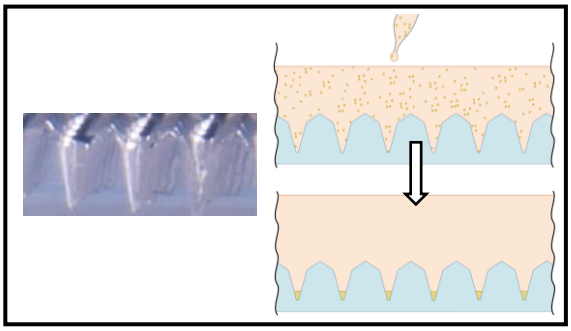
Component	Qty	abc biopply P/N
3D CoSeedis™ Chip680, 1 chip for 680 organoids in 1xPBS w/o Ca^{2+}/Mg^{2+}	1	C680
3D CoSeedis™ Chip680-6, 6 chips for 680 organoids in 1xPBS w/o Ca^{2+}/Mg^{2+}	1	C680-6
3D CoSeedis™ Chip680-12, 12 chips for 680 organoids in 1xPBS w/o Ca^{2+}/Mg^{2+}	1	C680-12
Spatula for 3D CoSeedis™ C680, sterile	1	SPT-V0919

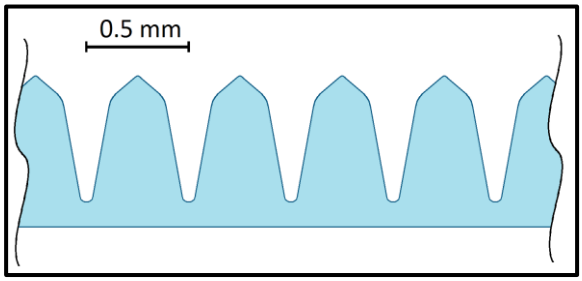
Introduction

3D CoSeedis™ Chip680, a 3-dimensional (3D) agarose-based co-culture system from ***abc biopply*** can be used in standard cell culture plates (24-well plates, see below). The system enables easy and economic seeding, cultivation and evaluation of extra small 3D cell aggregates. In addition, it serves as an array for a standardised colony formation assay (CFA), including read-out (for further information, visit our web site: www.biopply.com).

The system is intended for research only.

The agarose matrix forms a framework of conically shaped cavities and is seeded with cells in suspension. Since agarose repels and prevents cells adhesion, they sediment to the bottom of each cavity where they form hundreds of aggregates per chip.

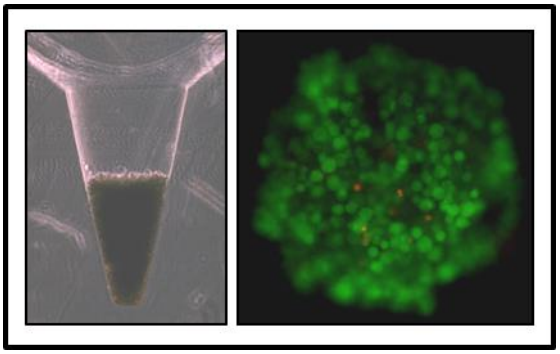




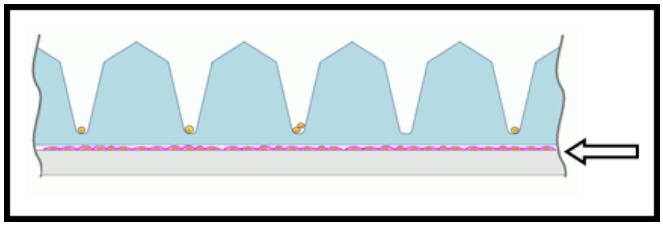
3D CoSeedis™ Chip680

There is currently one 24-well plate framework available: 3D CoSeedis™ Chip680 for 680 3D cultures.
In addition, ***abc biopply*** offers two 6-well plate frameworks (P/N: C200 and C880; see protocol 3DCS_SP_C200_C880).

Agarose is permeable to gases, nutrients and metabolites. Feeding of the cells therefore happens mainly via diffusion. Similarly, cytokines, dyes and other small molecules can easily diffuse through the matrix.



PI/FDA staining to label living and death cells in chip



Depending on the type of experiment, different cell types can be seeded and co-cultured in the cavities of the chip. Furthermore, it is possible to co-culture the cells in the chip with underlying adherent feeder cells or particles (arrow). Those may provide growth and other factors (via diffusion) while being physically separated from the cells in the chip.

Technical details

The chips are provided in 1xPBS w/o Ca^{2+}/Mg^{2+}.

	3D CoSeedis™ Chip680
distance between the centre of 2 microwells	0.5 mm
no. of microwells/cm²	400 microwells/cm²
total no. of microwells/chip	~ 680 microwells
diameter of microwell at the bottom	46 µm

Required material

- 24-well plates with a well diameter of at least 15.5 mm
  Note: the following plates show the appropriate dimensions and are suitable for 3D CoSeedis™: Corning® Costar®, Falcon®, greiner bio-one CELLSTAR®, Nest®.
- Spatula for 3D CoSeedis™ C680 (P/N: SPT-V0919)
  
  Sterilisation via autoclave or immersion in 70% Ethanol (non-denatured)
- Cell strainer (pore size 40 µm)
- Hemocytometer or alternative cell counting device
- Trypsin/EDTA (P/N : PEL-PB-LU-002-0100)
  Alternative: Accutase (P/N : PEL-PB-PAACUTASE)
- Vacuum suctions device and fitting pipettes
- Serological pipettes and according pipetting device
- Cell culture growth medium incl. supplements
  Note: we recommend 2.5% serum for 3D cultures. Higher concentrations for clonal growth only.
- Centrifuge for microtiter plates
- Analysis tool for cell seeding and volumetric analysis
  (free download at: https://biopply.com/support/analysis-tool/)

Protocol

(1) Equilibration of agarose chip(s) in 24-well plates: 1¼ h

The agarose chip is delivered in single-packaged aluminium bag containing 1xPBS w/o Ca^{2+}/Mg^{2+}. Alternatively, bulk quantities of chips (P/N: C680-6 or C680-12) are delivered and stored in plastic beakers containing 1xPBS w/o Ca^{2+}/Mg^{2+}.
In order to create the proper environment for cells to grow in, the chip needs to be equilibrated before seeding:

(a) Add 1 ml of growth medium into a well of a fresh 24-well plate (see above for correct plate dimensions).
(b) Transfer chip into the medium. If equilibrating multiple chips, apply 1 chip per well. Use the spatula (P/N: SPT-0919).
(c) Centrifuge plate for 3 – 5 minutes at 200 g to remove air bubbles.
(d) Equilibrate chip(s) for at least 1 h (to overnight) in a 37°C, humidified incubator.
➢ Chip(s) will take up the colour of the medium.

(2) Plated cell number and cell suspension: ½ h

When choosing the number of target cells per microwell (cpm), those general guidelines apply:

- For cell types that grow well only in larger numbers per microwell, a multicellular start is recommended (e.g. HepG2, Hep3B).
- Cells that grow well in an anchorage-independent manner (which is the provided environment in 3D CoSeedis™ microwells) can be seeded from 1 cpm to more than a 1 000 cpm. However, since space is limited in the microwells of a 3D CoSeedis™ Chip680, we do not recommend do seed more than 500 cpm.
- This chip contains 400 cavities/cm^2. Each cm^2 will be seeded with 1 ml of cell suspension.
  ➢ To seed 100 cpm, we need 400 x 100 = 40 000 cells per 1 ml.
  ➢ Each well has a surface area of approximately 1.9 cm^2. Therefore, we need 1.9 x 40 000 = 76 000 cells in 1.9 ml of cell culture medium.

Cells/micro-well	Cells/ml medium	Comment	Cell suspension per chip
1	400	3D aggregation and growth will rely to a large extend on clonal growth. Good starting point for colony forming assay.	1.9 ml lower volumes will lead to a less homogeneous cell distribution across the chip.
5	2 000		
10	4 000		
100	40 000	Standard for radiation/chemo experiments with cancer cells.	
500	200 000	Medium change at least every 2nd day.	

(3) Prepare cells: ½ h

Please visit our web site (www.biopply.com) to download an excel spreadsheet to assist in cell dilutions.

(a) Detach cells from 2-dimensional (2D) flask/dish according to standard protocol (e.g. Trypsin/EDTA, P/N: PEL-PB-LU-002-0100).
(b) Pass cell suspension through cell strainer (pore size 40 µm).
(c) Determine cell density (cells/ml) by using a hemocytometer (or any other alternative cell counting device).
(d) Dilute cells in medium to the required density for seeding chips:
    - for colony forming assays and limited dilution experiments, use 10% serum
    - for all experiments with higher cell densities, use 2.5% serum

    Note: when aggregates should be cultured for prolonged periods of time, it is recommended to test even lower serum concentrations if not to dispense with serum altogether. In 3D CoSeedis™ Chips, cells tolerate much lower serum concentrations than in 2D conditions (autocrine supply of factors).

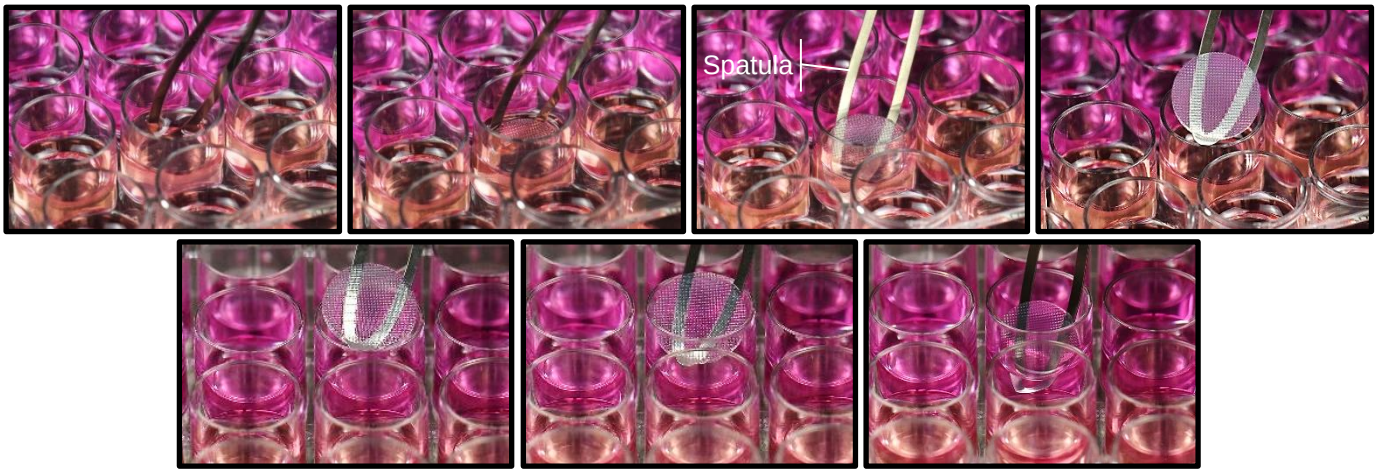
(4) Seeding cells: ½ h

(a) Remove equilibration medium from chip(s) only **after** the cell suspension is ready.
Important: after the equilibrium medium has been removed, each chip needs to be seeded with cell suspension within a few minutes. Long exposure of chip(s) to air will lead to precipitates.
(b) Add 1.9 ml of cell suspension/well (e.g. with 1 000 µl pipette).
Note: apply the suspension to the centre of each chip.
(c) After seeding, we recommend to leave the 24-well plate in the sterile cabinet for at least 20 minutes before transferring it to the incubator. This will allow most cells to settle down.
(d) Transfer the 24-well plate to incubator to allow cells to sediment completely. Do not disturb 24-well plate for at least an hour. Ideally, cells are allowed to settle down for 8 hours to overnight.

(5) 1st medium change: <¼ h

When changing the medium for the first time, all *chip(s) are transferred into a new 24-well plate*. This will get rid of adherent cells that attached to the plastic of the 24-well plate when cells were initially seeded.
Cell densities for clonal cell growth do normally not require media changes. Nevertheless, transferring the chip(s) to a new 24-well plate after seeding is recommended.

(a) Add 1 – 2 ml of fresh medium to each well of a new 24-well plate. The volume of medium may have to be adjusted according to the number of cells seeded and their metabolic activity.
(b) Lift chip(s) from the old 24-well plates by using the spatula for 3D CoSeedis™ C680 and transfer them into the medium of the new 24-well plates (eventually, push chip(s) down with spatula – careful!):



(6) Subsequent medium changes: **<¼ h**

For subsequent medium changes, it is not required to transfer chip(s) to a new 24-well plate.

(a) Remove old medium by using a vacuum suction device.
Note: careful not to damage the chip and not to lose any cells!
(b) Add 1 – 2 ml of fresh medium to each well of the 24-well plate. Do apply medium to the side wall of each well.
Note: careful to not flush the cell aggregate out of their cavities!

(7) Harvesting of organoids: **<¼ h**

It is possible to harvest 3D organoids for further processing such as:

- Single cell analysis
- DNA, RNA or protein extraction
- Re-embedding of 3D structures in alternative matrices
- High-throughput testing of individual 3D organoids
- Etc.

To harvest 3D structures:

1. Lift chip(s) containing the organoids from the corresponding well as depicted above (step 5 (a) and (b)).
2. Flip it upside-down and put it back into a well containing either medium or any other physiological buffer for organoid collection (e.g. 1xPBS w/o Ca^{2+}/Mg^{2+}).
3. Centrifuge at 300 g for 30 seconds.
4. Lift off and discard empty chip(s).
(Beforehand, make sure no 3D organoids are left behind in the chip – an additional centrifugation step may be required otherwise).
5. Organoids in the medium or buffer may now be collected for further processing using a serological pipette.

Cell type behaviour in the 3D CoSeedis™ cell culture platform

Name	Description	Type of 3D construct Sp = compact spheroids Lo = loose aggregates	Clonal growth of single cells without additional ECM*
A-549	Human lung adenocarcinoma	Lo	not tested
B16	Murine melanoma	Lo	+
BT-474	Human breast carcinoma	Sp	not tested
FaDu	Human hypopharyngeal carcinoma	Lo	not tested
GBM4	Human glioblastoma	Lo	+
Hep3B	Human hepatocellular carcinoma	Lo	(+)
HepG2	Human hepatocellular carcinoma	Lo	(+)
HT-29	Human colorectal adenocarcinoma	Sp	+
Huh-7	Human hepatocellular carcinoma	Lo	(+)
HuT-78	Human T lymphocyte	sedimented suspension cells	not tested
LnCap	Human prostate cancer	Lo	not tested
MCF-7	Human mammary cancer	Lo	-
MD-MBA-231	Human mammary cancer	Lo	-
Mesenchymal Stroma-Zellen (MSC)	Primary human cells from bone marrow and adipose tissue	Sp	-
MiaPaCa-2	Human pancreatic adenocarcinoma	Lo	+
MTPa	Rat mammary cancer	Sp	+
OE-19	Human esophageal adenocarcinoma	Sp	+
Panc-1	Human pancreatic adenocarcinoma	Lo	(+)
PC3	Human prostate cancer	Lo	-
PSN-1	Human pancreatic adenocarcinoma	Lo	(+)
Raji	Human B lymphocyte	sedimented suspension cells	not tested
Snu	Human hepatocellular carcinoma	Lo	(+)
T 47D	Human mammary cancer	Sp	(+)
U251	Human glioblastoma	Sp	+
U266	Human multiple myeloma	Lo	-
WiDr	Human colorectal adenocarcinoma	Sp	+

*) clonal growth of adhesion-dependent cells can be stimulated through the addition of ECM particles.

<u>Note:</u> the following cells show poor cell growth under standard conditions (i.e. without feeder support and other adaptations):

- *MaMel 63a*
- *MaMel 47*
- *FLO-1*
- *OAC-P4C*