Catalogue No. AMS.CSR-ABC-KIT

Alginate 3D Cell Culture Kit

Transformed cells, such as tumor cells, have the characteristic feature of anchorage- independent growth, unlike normal cells. Some normal cells, such as chondrocytes, are also capable of anchorage-independent growth, and the phenotypic expression of these cells is known to be stronger compared with monolayer cultures. Soft agar culture is a method in which cultures are grown with cells suspended in soft agar gel, and has been used conventionally as a method to detect the ability of cells to undergo anchorage-independent growth. As agar solidifies on cooling, the temperature must be maintained at approx. 37°C while preparing the seed culture plate. Also, since special reagents are required when harvesting the cells in the gel, the resulting culture is not suitable for analysis of cell function.

In contrast, alginate, which is an anionic polysaccharide derived from cell walls of brown algae, form a gel in the presence of calcium and liquefy to a solution upon addition of a calcium chelating agent. Alginate gel has been a choice for three-dimensional (3D) cell culture because only cultured cells can be easily harvested.

The Alginate 3D Cell Culture Kit is a convenient, easy-to-manufacture kit optimized to produce alginate gel beads. This product has been used to develop successful 3D cell culture systems for a range of different cells types including tumor cells and chondrocytes.

1) Kit Components:

1. Sodium alginate solution (25 mL ×1 bottle, sterile)
2. Calcium chloride solution (100 mL ×2 bottles, sterile)
3. Sodium citrate solution (100 mL ×1 bottle, sterile)
4. Plastic flexible needle (4 pieces, sterile)
5. 24-well plate (4 pieces, for suspension culture, sterile)

2) Materials and Instruments Required, but Not Included:

1. Physiological saline (sterile)
2. 22G Hypodermic needle [e.g., Terumo, product code (NN-2238R)]
3. 5 mL Syringe [e.g., Terumo, product code (SS-05SZ)]
4. Magnetic stirrer and magnetic stir bar (sterile)*
5. Specimen cup or 100 mL beaker (sterile)*
6. Spatula (sterile) [e.g., Coning, product code (3003)]

*Not required if alginate beads are directly prepared in a 24-well plate (see Method 2, below).

3) Preparation of Alginate Beads:

Methods for preparation of alginate beads are illustrated below. All procedures should be carried out in a biological safety cabinet under aseptic conditions. Be sure to allow the solution included in the kit, saline, and media to equilibrate to room temperature before use.

Method 1 (Method of preparing the beads directly in a 24-well plate)

(1) Dispense 2 mL calcium chloride solution to each well of the 24-well plate provided.
(2) Harvest cells using enzymes such as trypsin, and prepare cell pellet (containing 1×10^6 cells) into a 15 mL tube.
(3) Dispense 5 mL sodium alginate solution into the 15 mL tube and mix well with a pipette to ensure a homogeneous cell suspension (e.g., 2×10^5 cells/mL).
(4) Attach the plastic flexible needle to a 5-mL syringe and aspirate the cell suspension into the syringe.
(5) Remove the plastic flexible needle from the cell suspension syringe and attach a 22G needle instead.
(6) Hold the cell suspension syringe in an upright position over the 24-well plate and add 10 drops of cell suspension into each well containing calcium chloride solution at about one drop per second (1 drop is ca. 10 ul). Position the tip of the needle 3-4 cm above the liquid surface of calcium chloride and drop the cell suspension (**Figure 1**). If the tip is positioned too close to the liquid surface, alginate will form a big aggregate of gel.
(7) When finished, cover the plate with a lid, and leave it at room temperature for 10 min.
(8) Check if the alginate beads have coagulated (**Image 1**). The beads should appear completely white by visual observation; the center of the bead should also be white. We recommend that the beads be placed against a black background for better visualization.

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(9) Remove the calcium chloride solution from the well using a manual pipette. Tilt the pipette slightly, and gently remove the solution while allowing the tip to touch the bottom of the well, so that the solution can remove through the tip without aspirating the beads.
(10) Dispense 2 mL saline solution to each well, and let stand at room temperature for 15 min.
(11) Remove the saline solution from each well in a similar manner of (9). Dispense 2 mL medium to each well, and let stand at room temperature for 15 min.
(12) Remove the medium from each well in a similar manner of (9). Dispense an additional 1–2 mL medium, add test substances into the culture medium and let start the culture.
(13) Change the medium every few days, if needed, until a good formation of colonies is achieved in each well.

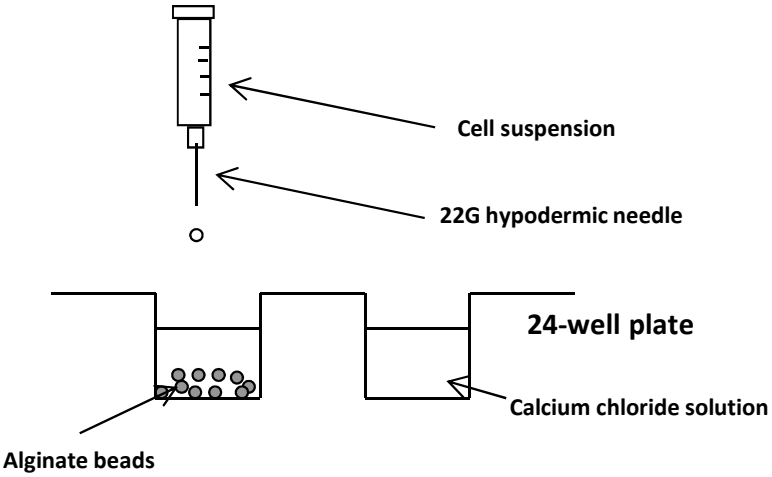

Figure 1

Image 1

Method 2 (Method of preparing all beads at once using a specimen cup)

(1) Put a sterile stir bar in a sterile specimen cup (or a 100 mL beaker) and dispense 50 mL calcium chloride solution. (Preparation of beads is possible without using a stirrer, but better results can be obtained if the solution is stirred.)
(2) Harvest cells using enzymes such as trypsin, and prepare the cell pellet (containing 1×10^6 cells) into a 15 mL tube.
(3) Dispense 5 mL sodium alginate solution into the 15 mL tube and mix it well with a pipette to ensure a homogeneous cell suspension**.**
(4) Attach the plastic flexible needle to a 5-mL syringe and aspirate the cell suspension into the syringe.
(5) Remove the plastic flexible needle from the cell suspension syringe and attach a 22G hypodermic needle.
(6) Set the specimen cup containing the calcium chloride solution on the stirrer, and rotate the stir bar to mix the solution.
(7) Hold the cell suspension syringe in an upright position over the specimen cup (Figure 2). Add the cell suspension into the calcium chloride solution containing cup at about two drops per second. Position the tip of the needle 3 cm or higher above the liquid surface of calcium chloride.
(8) When finished, let the beads stand for 10 min without turning off the stirrer. Check if the alginate beads have coagulated. The beads should appear completely white by visual observation; the center of the bead should also be white. We recommend that the beads be placed against a black background for better visualization.
(9) Remove the calcium chloride solution from the cup using a manual pipette. Tilt the 25 mL pipette slightly and gently remove the solution while allowing the tip to touch the bottom of the cup so that the solution can remove through the tip without aspirating the beads.
(10) Dispense 100 mL saline solution to the cup and mix for 10 min (or let stand for 15 min).
(11) Remove the saline solution from the cup in a similar manner of (9). Dispense 100 mL medium to the cup and mix for 10 min (or let stand for 15 min).
(12) Dispense 1–2 mL medium to each well of a 24-well plate.
(13) Remove the medium from the cup in a similar manner of (9).
(14) Use a sterile spatula to scoop 10 alginate beads from the cup and place each bead into a separate well of the 24-well plate.

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(15)Add test substances into the culture medium to start the culture.
(16)Change medium, if necessary, until a good formation of colonies is achieved in each well.
(For a method of medium changes, see Changing the Medium, below.)

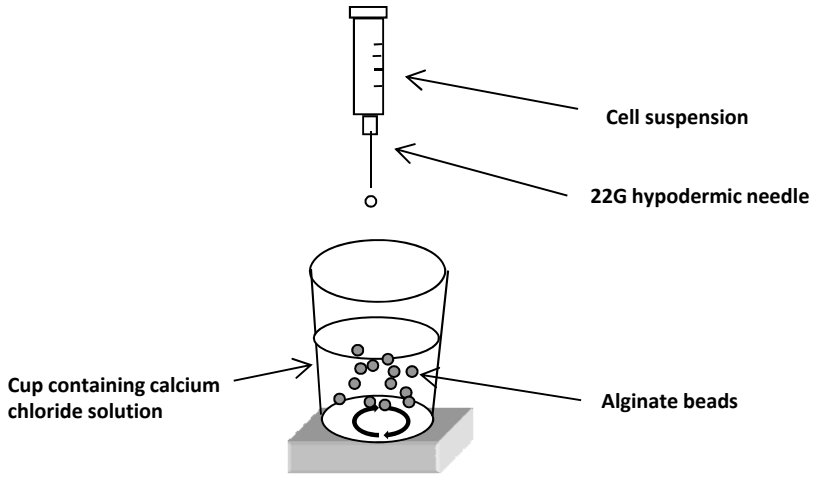

Figure 2

4) Changing the Medium:

(1) When transferring the solution, leave the pipette tip attached to the bottom of well and remove the medium slowly at a slight angle against the inside wall.
(2) Add fresh medium and continue to culture

5) Cell Recovery Method from Alginate Beads:

Method 1 (Method of recovery of cells from only a part of the 24-well plate)

(1) Remove the medium from the well using an aspiration pipette
(2) Transfer the alginate beads to 1.5 mL tubes using a sterile spatula.
(3) Add 1 mL sodium citrate solution to each tube, and mix for 5–15 min at room temperature to dissolve the alginate beads.
(4) Centrifuge at approximately 300 Xg for 3 min.
(5) Remove the supernatant, and harvest the cells as a precipitated pellet.

Method 2 (Method of recovery of cells from the entire 24-well plate)

(1) Remove the medium from the entire well using an aspiration pipette.
(2) Add 1 mL sodium citrate solution to each well, and mix for 5-15 min at room temperature to dissolve the alginate beads.
(3) Transfer the alginate solution to 1.5 mL tubes and centrifuge at approximately 300 ×g for 3 min.
(4) Remove the supernatant, and harvest the cells as a precipitated pellet

6) Precautions for Use:

1. Ensure careful handling of the instruments, including the syringe needle, during cell culture procedures (e.g., removal and addition of cell suspension).
2. Similar to soft agar cultures, certain types of cells may not be suitable for 3D cell culture using the alginate gel system.
3. We recommend the use of a manual pipettes during the course of treatment carried out with alginate beads (e.g., washing of the beads and exchanging the medium). Using a manual pipette will enable slow, controlled dispensing, while using a Pasteur pipette may suck up not only the solution but also the beads.
4. Cells falling out from alginate beads may grow on the 24-well suspension culture plates after long-term usage. In such cases, we recommend that you transfer the alginate beads to a new plate.
5. This product is intended for laboratory research use only and is not to be used for any other purpose, including clinical uses.

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7) References and application example:

1. Chemotherapy screening assay using 3-dimensional cell culture. *Cancer Lett,* 51,11-16, 1990.
2. Expression of a stable articular cartilage phenotype without evidence of hypertrophy by adult human articular chondrocytes in vitro. *J Orthop Res*. 16, 207-216, 1998.

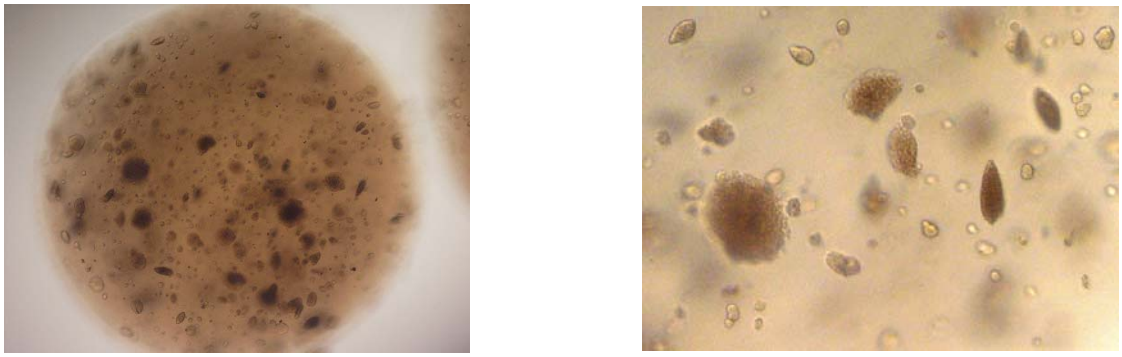

Image 2
HepG2 cell cultured in alginate beads for 9 days（left：low magnification、right：high magnification）

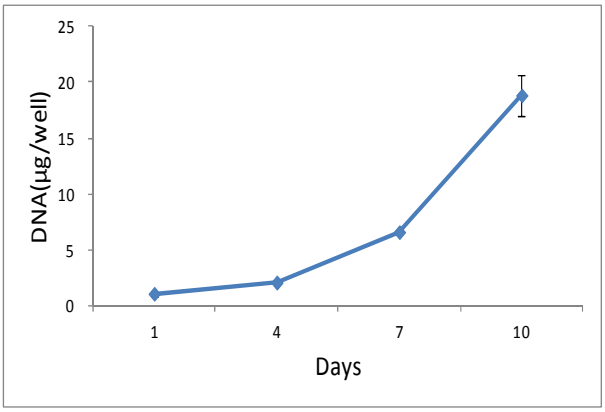

Figure 3
Growth curve of HepG2 cells cultured in alginate beads（Mean±SD, n=3）

8) Products:

Product code	Product name	Quantity	Remarks
AMS.CSR-ABC-KIT	Alginate 3D Cell Culture Kit	1 kit	Sterile, Store below 4°C, Do not Freeze
AMS.CSR-ABC-AL	Sodium alginate solution	25mL	Sterile, Store below 4°C Do not Freeze
AMS.CSR-ABC-CA	Calcium chloride solution	100mL	Sterile, Store below 4°C Do not Freeze
AMS.CSR-ABC-CI	Sodium citrate solution	100mL	Sterile, Store below 4°C Do not Freeze

For research use only, Not for diagnostic use.

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Application example of Alginate 3D Cell Culture Kit

1. Chondrocyte

Normal porcine chondrocytes prepared from articular cartilage of knee joint were cultured in alginate beads at $2x10^6$ cells/mL (in DMEM/F-12 containing 10%FBS, 100ng/mL IGF-I and 25μg/mL L-ascorbic acid) in the presence or absence of oversulfated chondroitin sulfate. At end of culture, the alginate beads were dissolved with sodium citrate solution and the cell pellet was digested with Pronase solution (1mg/mL). The DNA and collagen contents of the digested sample were determined using the Hoechst 33258 fluorescent dye method and measurement of hydroxyproline content, respectively (Tissue Eng Part A. 2010 May; 16:1575-84).

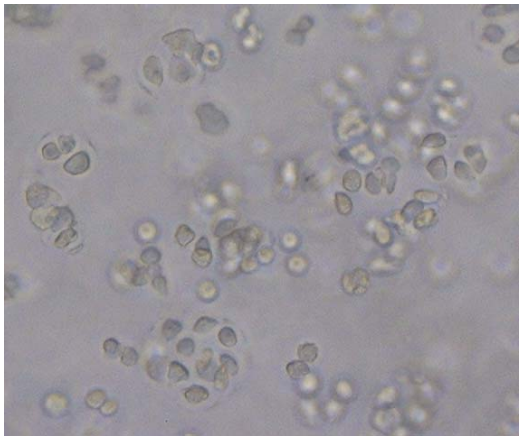

Image 1. Normal porcine chondrocytes cultured in alginate beads.

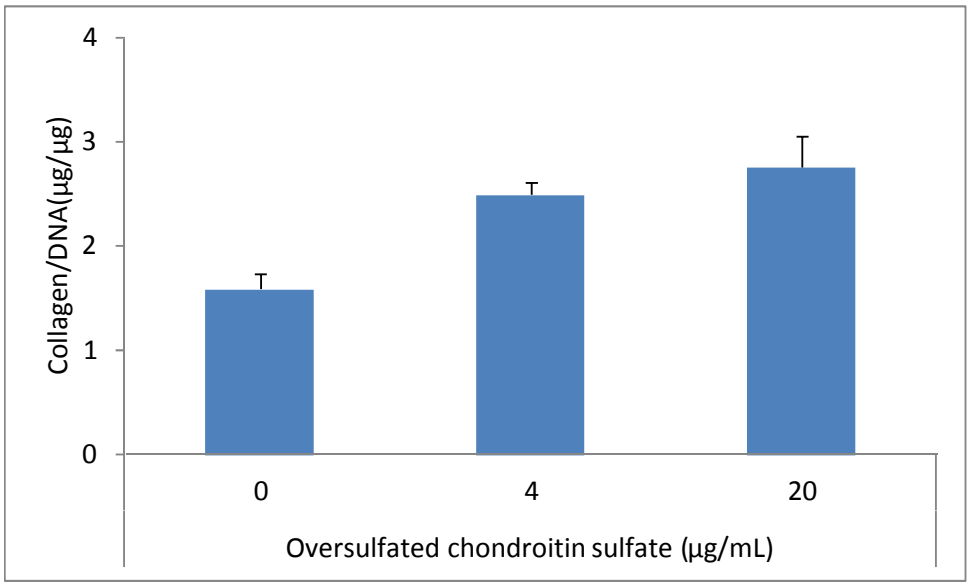

Figure 1. The effect of oversulfated chondroitin sulfate on the collagen production of chondrocytes in alginate beads.（at Day 6, Mean±SD, n=3）

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2. HepG2 cells

A human liver carcinoma cell line, HepG2 cells, was cultured in alginate beads (5×10^5cells/mL, 10beads/well, DMEM containing 10%FBS) for 9 days.

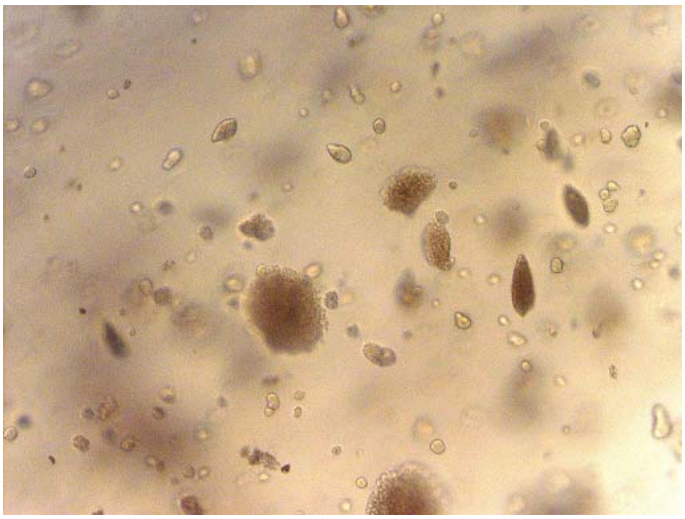

Image 2. HepG2 cells cultured in alginate beads.

HepG2 cells were cultured in alginate beads (1×10^5cells/mL, 10beads/well, DMEM containing 10%FBS). At end of the culture, the alginate beads were dissolved with sodium citrate solution and the cell pellet was digested with Pronase solution (1mg/mL). The DNA content of the digested sample was determined using the Hoechst 33258 fluorescent dye method.

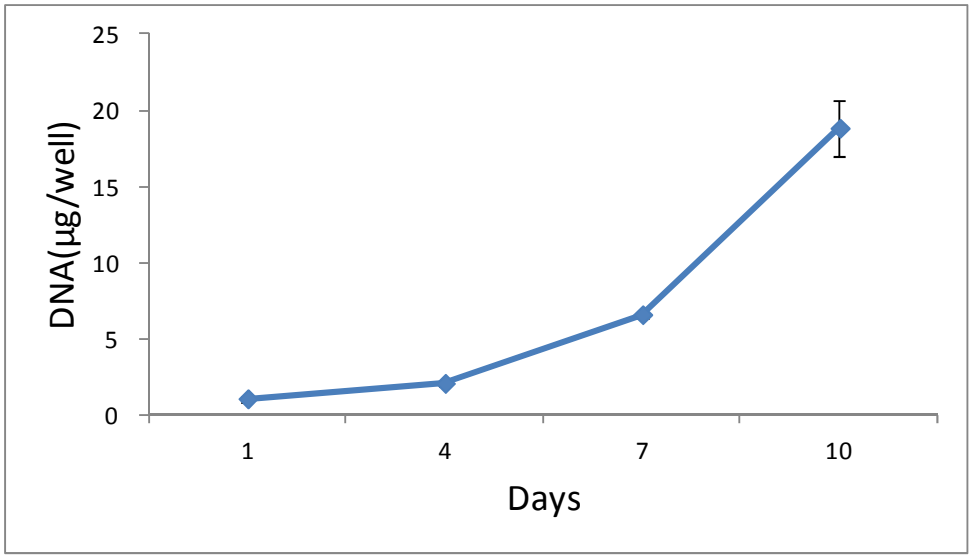

Figure 2. Growth curve of HepG2 cells cultured in alginate beads（Mean±SD, n=3）

HepG2 cells were suspended in alginate beads and cultured (2×10^5cells/mL, 10beads/well, DMEM containing 10%FBS). After 24 hours, antitumor drug, 5-flurouacil (5-FU), was added and further cultured for 6 days. At end of culture, the alginate beads were dissolved with sodium citrate solution and the cell pellet was digested with Pronase solution (1mg/mL). The DNA content of the digested sample was determined using the Hoechst 33258 fluorescent dye method.

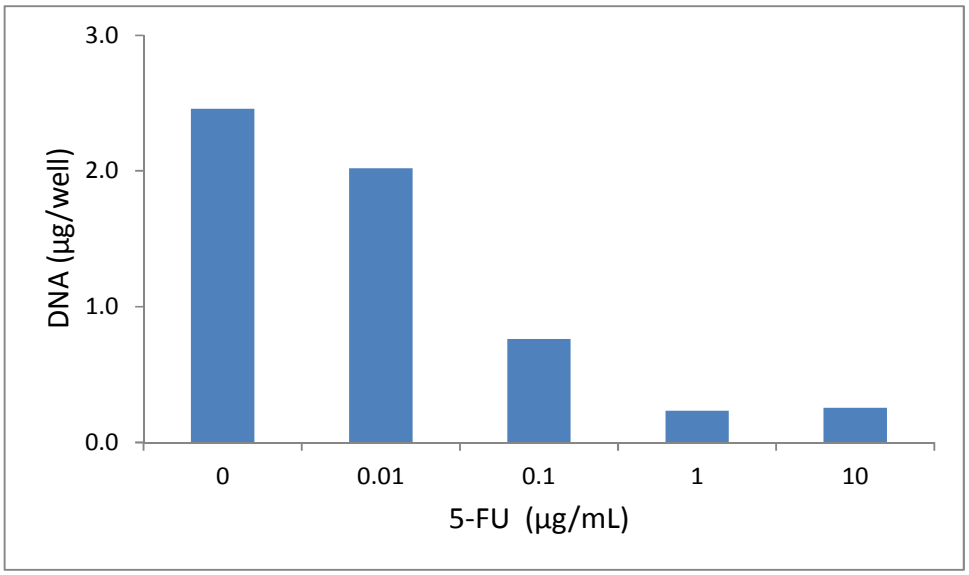

Figure 3. Effect of 5-FU on the growth of HepG2 cells in alginate beads.

3. Saos-2 cells

A human osteosarcoma cell line, Saos-2 cells, was cultured in alginate beads (2×10^5cells/mL, 10beads/well, DMEM/F-12 containing 10%FBS). At end of the culture, the alginate beads were dissolved with sodium citrate solution and the cell pellet was digested with Pronase solution (1mg/mL). The DNA content of the digested sample was determined using the Hoechst 33258 fluorescent dye method.

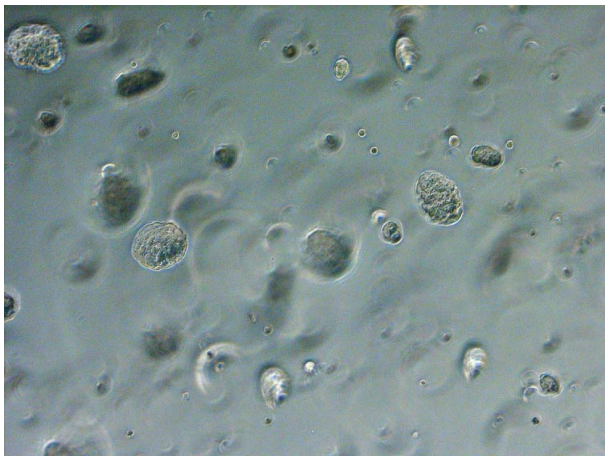

Image 3. Saos-2 cells cultured in alginate beads for 14days.

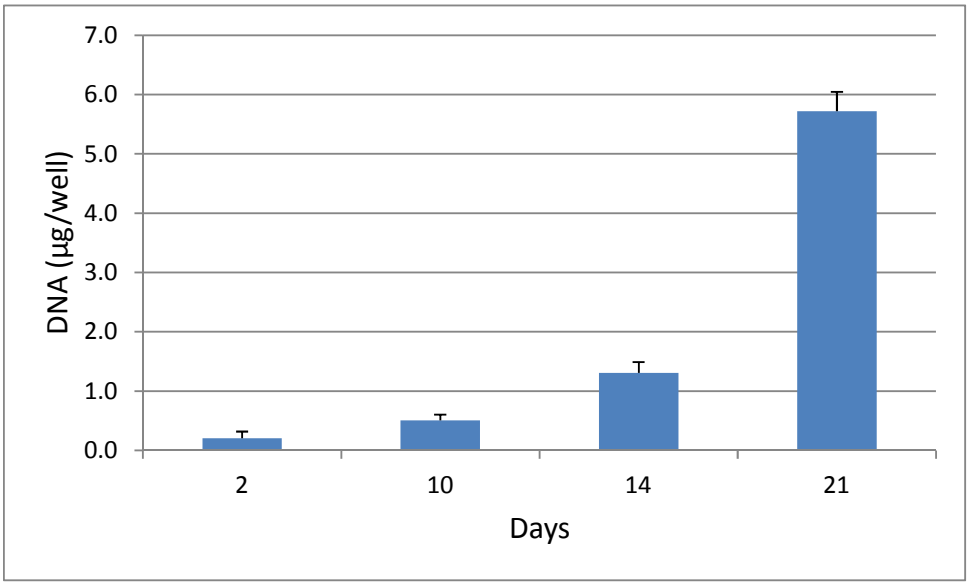

Figure 4. Growth of Saos-2 cells cultured in alginate beads（Mean±SD, n=3）

4. 3T3-L1 cells

A murine fibroblast cell line, 3T3-L1 cells, was cultured in alginate beads (2×10^5cells/mL, 10beads/well, DMEM containing 10%FBS). At end of the culture, the alginate beads were dissolved with sodium citrate solution and the cell pellet was digested with Pronase solution (1mg/mL). The DNA content of the digested sample was determined using the Hoechst 33258 fluorescent dye method.

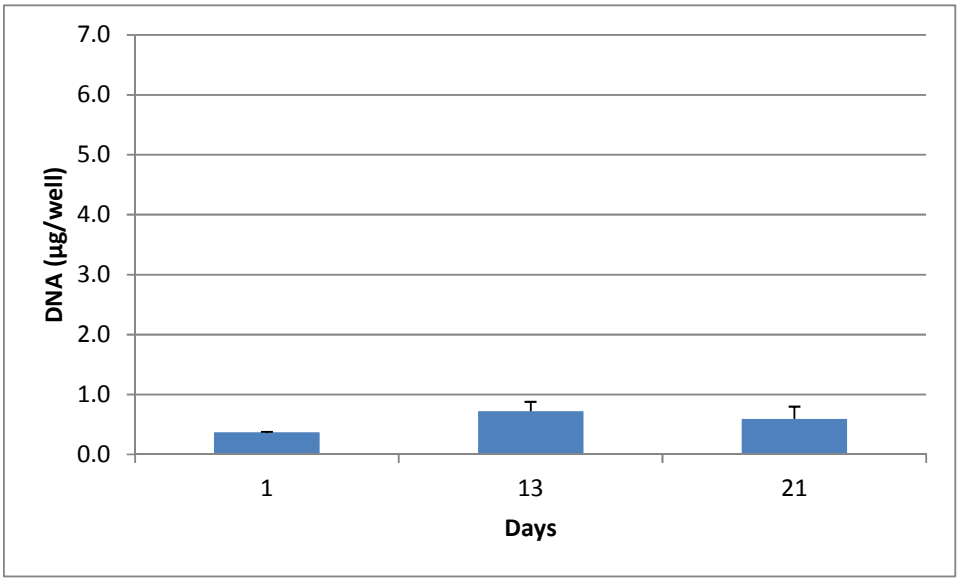

Figure 5. Growth of 3T3-L1 cells cultured in alginate beads（Mean±SD, n=3）
（3T3-L1 cells cannot growth in alginate beads）

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Alginate 3D Cell Culture Kit

A three-dimensional (3D) cell culture kit using alginate gel.

- ***Easy to produce alginate gel beads and harvest the cultured cells from gel.***
- ***Detect the ability of cells to undergo anchorage-independent growth like as soft-agar culture.***
- ***Suitable for 3D culture including tumor cells and chondrocytes.***

Kit Components:

Sodium alginate solution (25 mL)
Calcium chloride solution (100 mL ×2 bottles)
Sodium citrate solution (100 mL)
Plastic flexible needle (4 pieces)
24-well plate (4 pieces, for suspension culture)

Materials and Instruments Required, but Not Included:

Physiological saline (sterile)
22G Hypodermic needle
5 mL Syringe

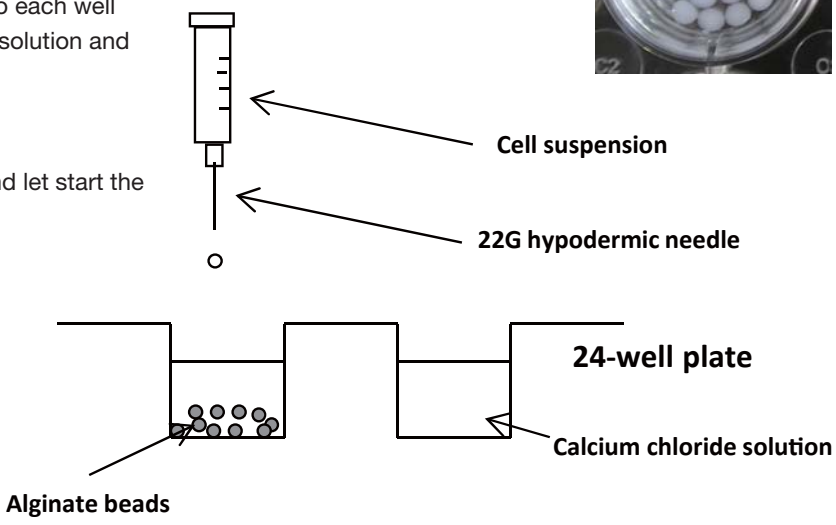
Preparation of Alginate Beads

(1) Prepare cell suspended alginate solution in 5mL syringe.

(2) Drop the cell suspension into each well containing calcium chloride solution and produce alginate beads.

(3) Wash alginate beads.

(4) Dispense culture medium and let start the culture.



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Alginate 3D Cell Culture Kit

A three-dimensional (3D) cell culture kit using alginate gel.

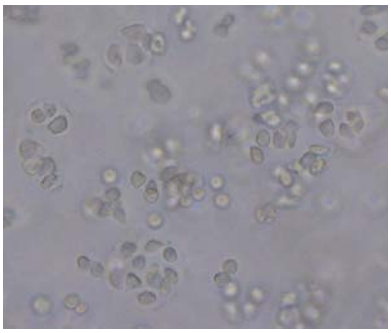
Cell Recovery from Alginate Beads

(1) Remove the medium from the well.

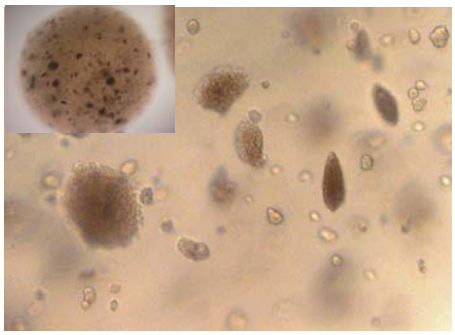
(2) Add sodium citrate solution and dissolve the alginate beads.

(3) Centrifuge and harvest the cells as a precipitated pellet.

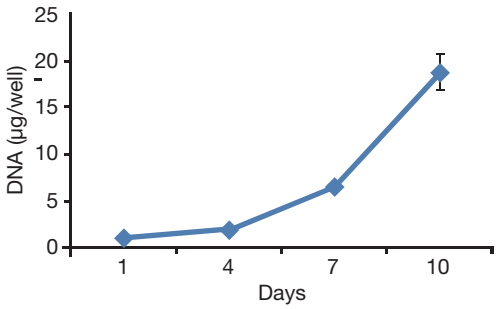
Reference Data



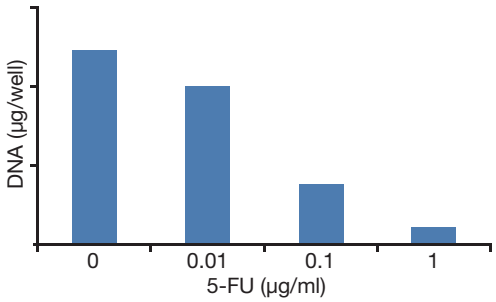
Porcine Chondrocytes cultured in alginate beads



HepG2 cell cultured in alginate beads



Growth curve of HepG2 cells cultured in alginate beads (Mean±SD, n=3)



Effect of 5-FU on the growth of HepG2 cells in alginate beads culture

Product name	Product code	Quantity	Remarks
Alginate 3D Cell Culture Kit	AMS.CSR-ABC-KIT	1 kit	sterile, store below 4°C, Do not Freeze
Sodium alginate solution	AMS.CSR-ABC-AL	25 mL	sterile, store below 4°C, Do not Freeze
Calcium chloride solution	AMS.CSR-ABC-CA	100 mL	sterile, store below 4°C, Do not Freeze
Sodium citrate solution	AMS.CSR-ABC-CI	100 mL	sterile, store below 4°C, Do not Freeze

For research use only. Not for diagnostic use.

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