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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

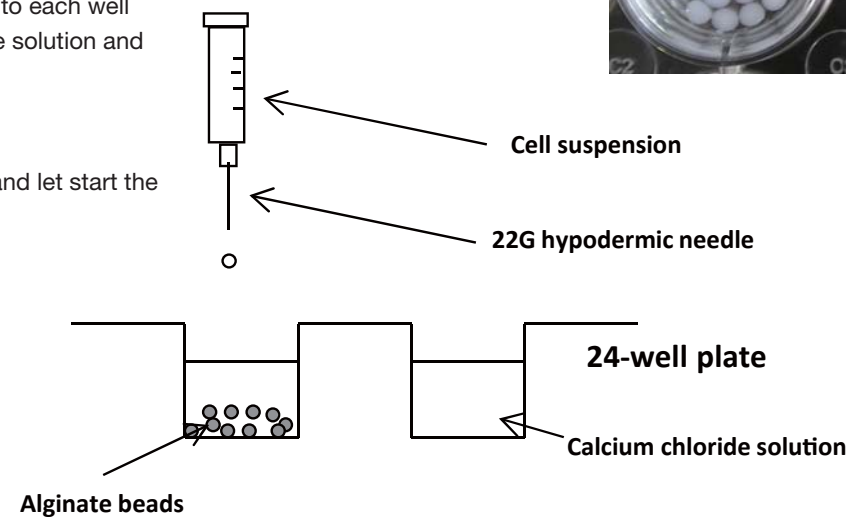
Preparation of Alginate Beads

(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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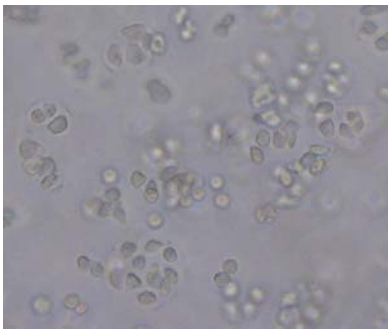
Cell Recovery from Alginate Beads

(1) Remove the medium from the well.

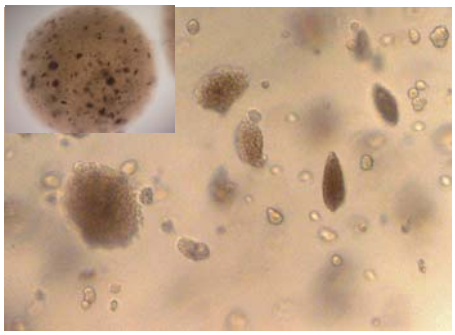
(2) Add sodium citrate solution and dissolve the alginate beads.

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

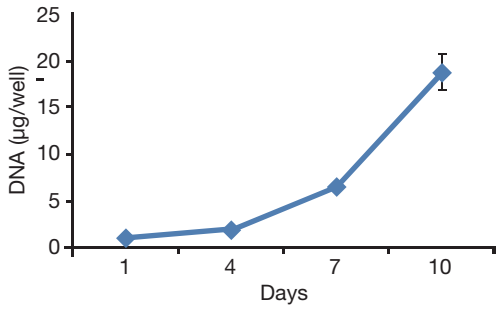
Reference Data



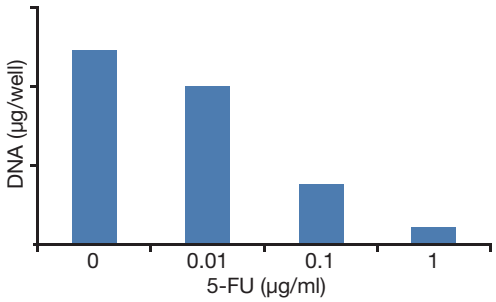
Porcine Chondrocytes cultured in alginate beads



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	CSR-ABC-KIT	1 kit	sterile, store below 4°C, Do not Freeze
Sodium alginate solution	CSR-ABC-AL	25 mL	sterile, store below 4°C, Do not Freeze
Calcium chloride solution	CSR-ABC-CA	100 mL	sterile, store below 4°C, Do not Freeze
Sodium citrate solution	CSR-ABC-CI	100 mL	sterile, store below 4°C, Do not Freeze

For research use only. Not for diagnostic use.

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).

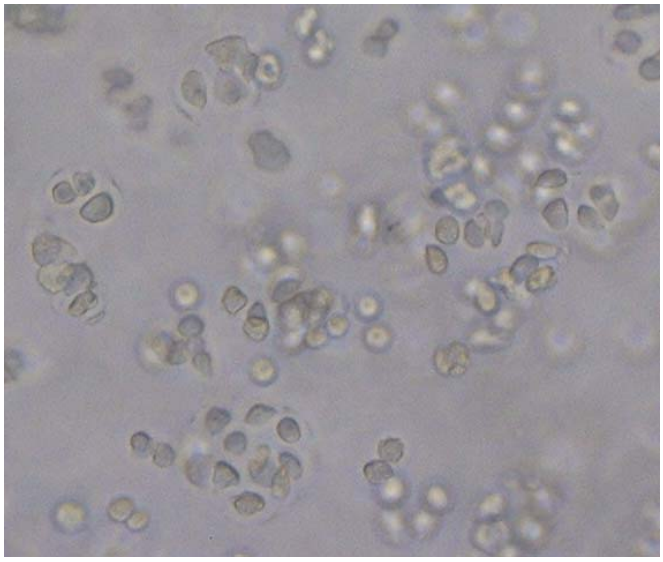


Image 1. Normal porcine chondrocytes cultured in alginate beads.

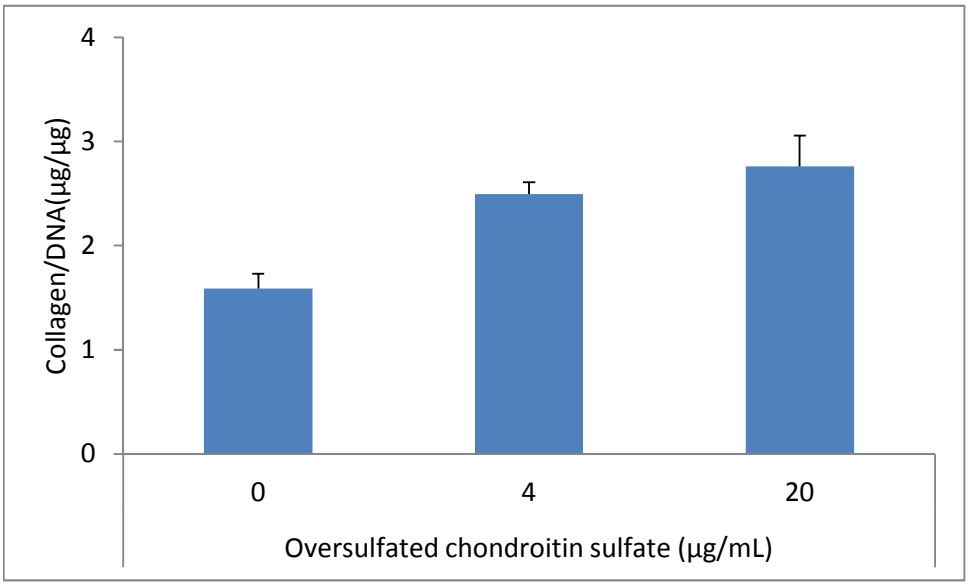


Figure 1. The effect of oversulfated chondroitin sulfate on the collagen production of chondrocytes in alginate beads.（at Day 6, Mean±SD, n=3）

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.

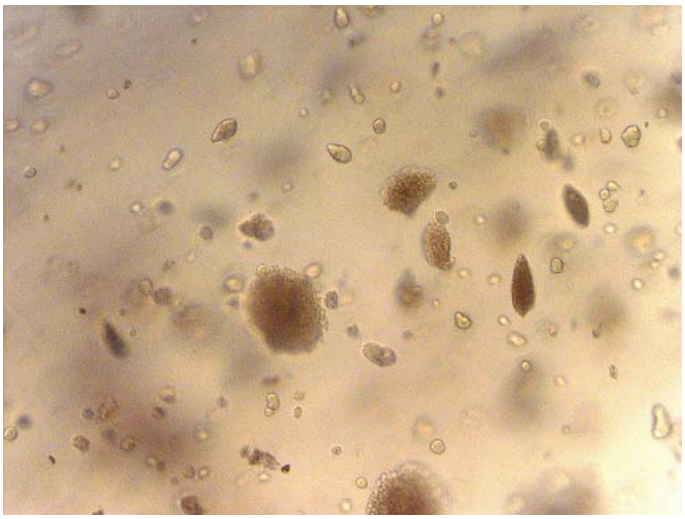


Image 2. HepG2 cells cultured in alginate beads.

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.

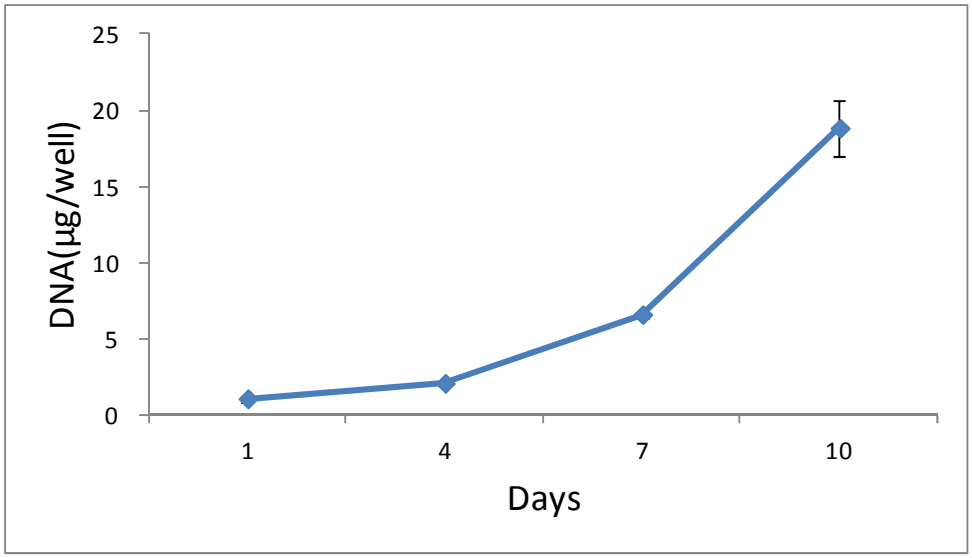


Figure 2. Growth curve of HepG2 cells cultured in alginate beads（Mean±SD, n=3）

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.

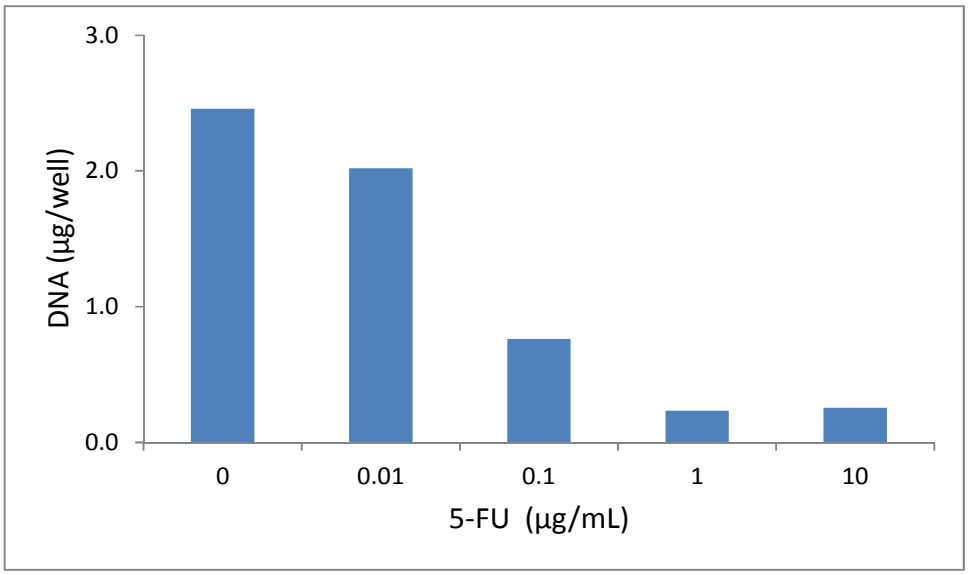


Figure 3. Effect of 5-FU on the growth of HepG2 cells in alginate beads.

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.

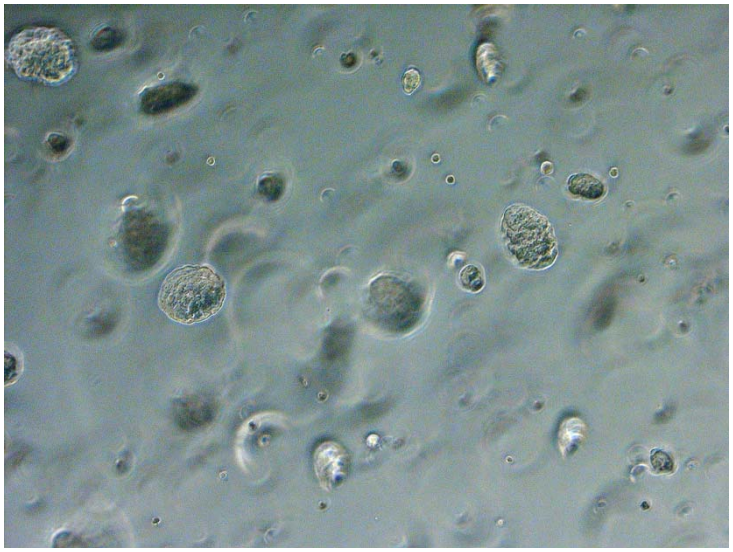


Image 3. Saos-2 cells cultured in alginate beads for 14days.

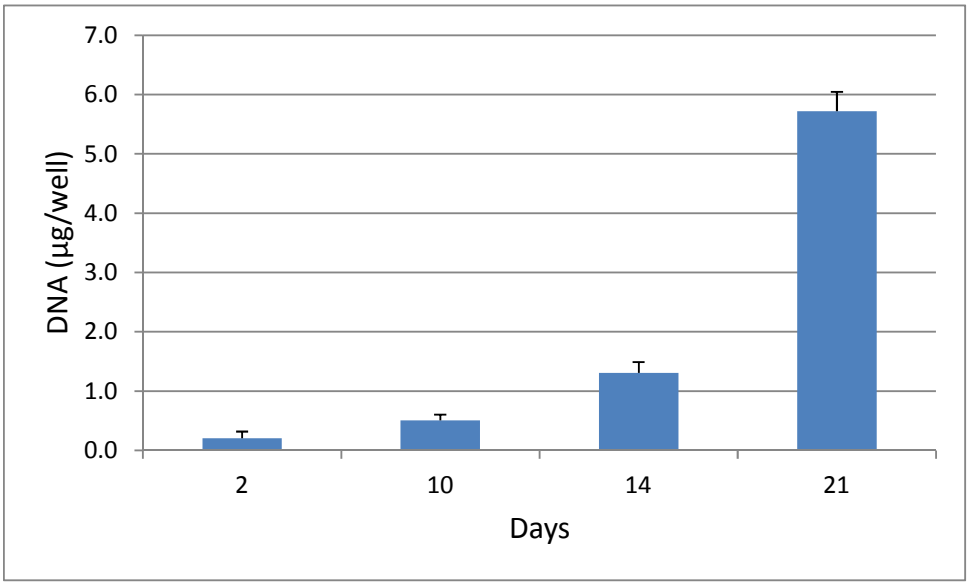


Figure 4. Growth of Saos-2 cells cultured in alginate beads（Mean±SD, n=3）

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.

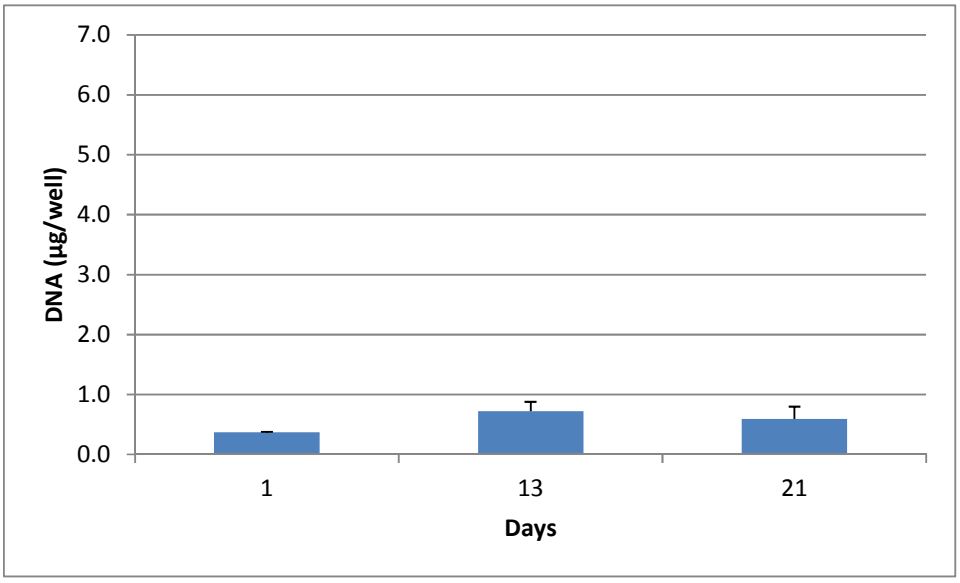


Figure 5. Growth of 3T3-L1 cells cultured in alginate beads（Mean±SD, n=3）
（3T3-L1 cells cannot growth in alginate beads）