Alginate 3D Cell Culture Kit

Cat. No. KITG-012 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	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.
Description	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

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Form	3D Cell Culture Kit
Applications	Three-dimensional (3D) cell culture for a range of different cell types including tumor cells and chondrocytes
Notes	Kit Components: Sodium alginate solution (25 mL × 1 bottle, sterile) Calcium chloride solution (100 mL × 2 bottles, sterile) Sodium citrate solution (100 mL × 1 bottle, sterile) Plastic flexible needle (4 pieces, sterile) 24-well plate (4 pieces, for suspension culture, sterile) Physiological saline (sterile) 22G Hypodermic needle 5 mL Syringe Magnetic stirrer and magnetic stir bar (sterile)* Specimen cup or 100 mL beaker (sterile)* Spatula (sterile) *Not required if alginate beads are directly prepared in a 24-well plate (see Method 2, below). Materials Required but Not Supplied: Instruments and materials not listed in the kit components. 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 µL). Position the tip of the needle 3-4 cm above the liquid surface of calcium chloride and drop the cell suspension. 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. 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 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. 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. 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. (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.) 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. Cell Recovery Method from Alginate Beads: Method 1 (Method of recovery of cells from only a part of the 24-well plate):

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	(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 × g 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. Precautions for Use: Ensure careful handling of the instruments, including the syringe needle, during cell culture procedures (e.g., removal and addition of cell suspension). Similar to soft agar cultures, certain types of cells may not be suitable for 3D cell culture using the alginate gel system. We recommend the use of a manual pipette 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. 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. Statement: This product is intended for laboratory research use only and is not to be used for any other purpose, including clinical diagnosis.
Usage	For Research Use Only

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