Seevix Material Sciences
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Product Working Protocol

SpheroSeev

Storage: Store SpheroSeev at 4^{0}C after opening.

Intended Use

SpheroSeev (Seevix spidersilk) is a reagent used for improving the viability, function and overall health of cell spheroid cultures. Seevix spidersilk fibers are supplied as a concentrated stock suspension to supplement cell culture media. The fibers are easy to handle and can be incorporated into spheroids by any spheroid generation technique as long as the spheroid-forming cells are suspended with the fibers prior to spheroid formation.
Fiber concentrations suggested below may need further optimization by the researcher depending on the type of cells and desired experimental conditions.
This product is intended for research only. Please consult safety data sheet before use.

Procedure

Cell Seeding Procedure (see Figure 1)

1. Calculate the desired cell concentration for 3D cell seeding and the appropriate volume of SpheroSeev to be added to the cells. We recommend seeding 100-3000 cells/spheroid in a 96-well plate supplemented with 0.2-0.6 ng of SpheroSeev/cell. To avoid medium evaporation, it is recommended to seed the spheroids only in 60 (of the 96) wells at the center of the plate and to fill the outer wells with 200µL of PBS.

   Example: For seeding 1 spheroid of 1000 cells in a 96-well plate at 0.5 ng SpheroSeev/cell:
   Media: 100- 200µL
   Cells: 1000 cells (volume depends on concentration of the stock)
   SpheroSeev: 1000 cells*0.5 ng/cell = 0.5 µg of fibers

2. Pre-warm the complete growth media in a 37^{0}C water bath.
3. Remove cells of interest from the culture vessel by trypsinization.
4. Determine the total number of cells and percent viability using a hemocytometer and Trypan Blue exclusion, or an automated cell counter.
5. Add SpheroSeev to the appropriate pre-warmed culture medium to achieve the desired concentration and mix well. Add cells to the mixture and mix well by gently pipetting up and down several times.
6. Dispense the cell-SpheroSeev mix into each well of the plate (200 µL/well in a 96 well plate).
7. Place the seeded plate in a 37^{0}C, 5% CO_2 humidified incubator.

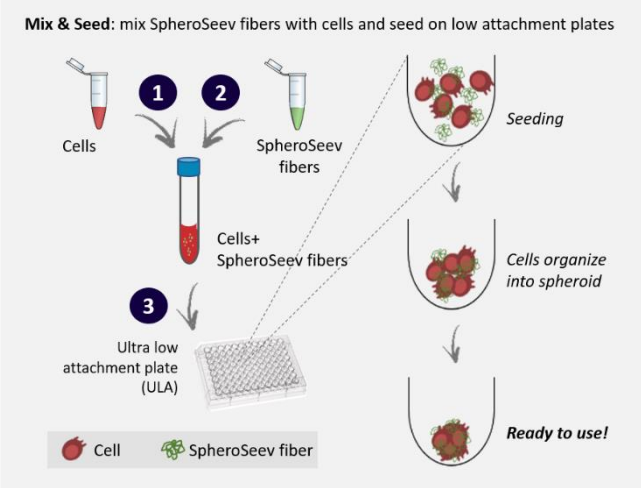

Figure 1: Fibers and trypsinized cells are mixed and seeded on an ultra-low attachment plate. During the following days, cells form aggregates containing trapped fibers and eventually establish cell-cell interactions to form stable spheroids.

Maintenance of Cells Cultured with SpheroSeev

Different cultures require different frequencies of medium replacement, depending on cell type, cell number, cell size and population doubling rate. We suggest using the same media exchange frequency as you normally use with your 3D cell cultures.

Time required for the assembly of spheroids is cell type dependent. For most cells, 24-72 hours are sufficient to form a single, stable spheroid. Start changing the medium of the culture as soon as the cells have formed a stable spheroid. Otherwise, delay the first medium change.
If cells were seeded in 100µl medium/well, add 100µl medium once a stable spheroid has formed,

The life span of the culture will also depend upon the cell type.

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Medium Replacement Procedure

1. Pre-warm the growth media in a 37^{0}C water bath.
2. (**Only for proliferating cells**) Prepare the maintenance medium by adding 2µg SpheroSeev per 1mL. Concentrations may need further optimization by the user. It is recommended to replace the medium if there are no excess free fibers in the medium (fibers should be visible under a light microscope - see Figure 2).
3. Carefully remove 100 µL/well of the spent media.
4. Gently add 100µL* fresh medium/well.
5. Return plate to a 37^{0}C, 5% CO_2 humidified incubator.

*For cultures kept for 2 weeks or longer, add 110 µL/well to compensate for medium evaporation.

Materials and Equipment

Material Provided

1 mg/mL SpheroSeev

Required Materials and Equipment Not Provided

Culture media (according to the cell type)
U-shaped ultra-low attachment plate
(or alternative equipment required for other spheroid generation technique)
Pipette & sterile pipette tips
Biosafety hood
Cell culture incubator

Note:

SpheroSeev fibers are visible under a light microscope (see Figure 2).

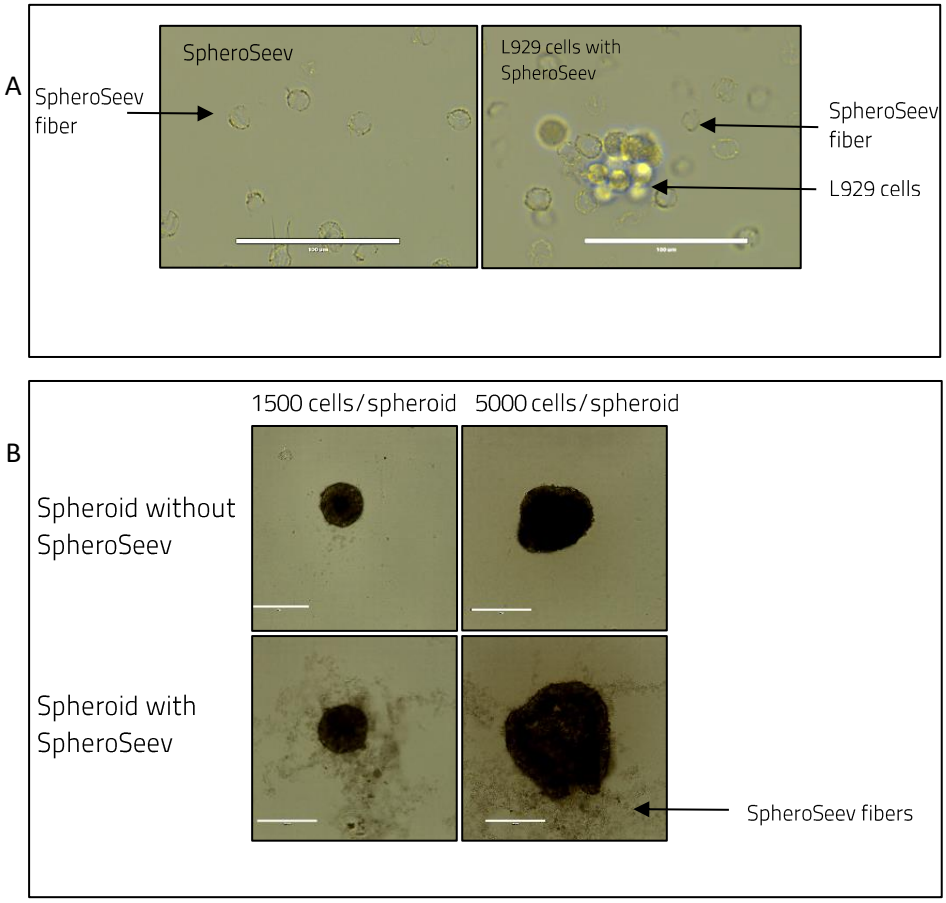

Figure 2:
A. SpheroSeev and L929 cells mixed with SpheroSeev.
B. Rat hepatocytes spheroids seeded in a culture plate, surrounded by fibers (day 4 post seeding).