GENProtocols

Protocol

Generating and maintaining uniform 3D spheroids in long term cultures

TRANSLATIONAL MEDICINE DRUG DISCOVERY

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Abstract

This instruction explains the basic operating procedure for generating and maintaining longterm three-dimensional spheroid cultures on PrimeSurface® 96 Slit-well plates. Instructions recommended here are the best operating protocols but depending upon your cell line and assay, some optimizations may be required.

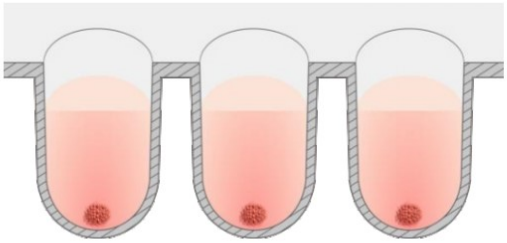
Introduction

Enhanced stem cell culturing in regenerative medicine cell culturing involves frequent media replacement to provide nutrition to growing cells. In a standard 96 well ultra-low cell attachment plate, media aspiration or dispensing has to be done extremely carefully to avoid disturbing the unattached spheroid, making this a time consuming operation. PrimeSurface 96 Slit-well Plate allows media handling and exchange for 96 well plates efficiently with one step dispensing or aspiration for all 96 wells, decreasing the pipetting time by over 80% while minimizing the risk of spheroid damage. The organoids grow more comparably sized in the slit well plate compared with either 10cm dishes or traditional 96 well plates. The interconnectedness of the wells ensures more biological consistency of the different wells. The benefit is the much faster time to feed cultures. Feeding also only requires a pipette while feeding traditional wells requires multichannel pipettes and media boats. The slit well plates reduce time of handling and cost, particularly for longterm cultures. The new design of ultra-low attachment 3D plates facilitate easy handling of media exchange without disrupting spheroid formation.

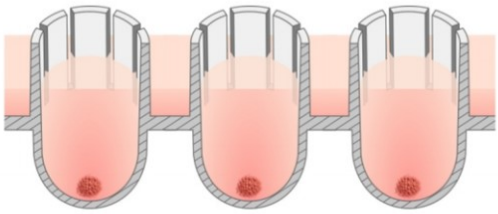
Benefits of slit-well plates for spheroid culture

Slit-well structure allows simultaneous delivery of cell culture medium to all 96 wells

Conventional product:
Each wells are independent

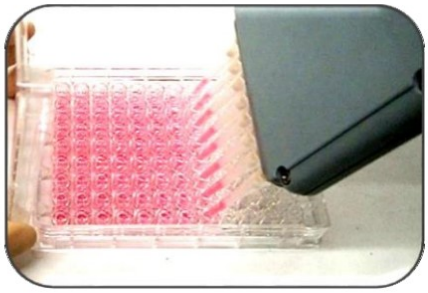


Slit-well Plate:
Medium is shared through slit-well design



Slit structure design for easy media exchange without being concerned about spheroid detachment or collapse

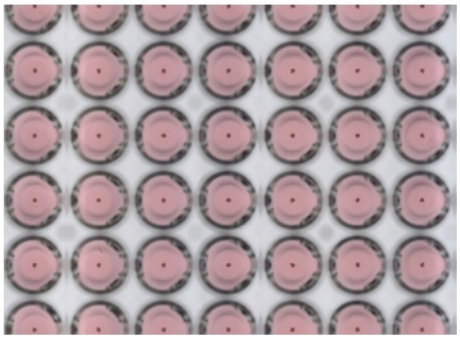
Feature 1: Minimize media exchange effort and time without disturbing spheroid formation



Conventional media exchange

Easy one step media exchange by tilting the plate and aspirating from the corner.

Feature 2: Generate and maintain uniform spheroids in long term cultures



Cell: HepG2 cell
Density: 1,000 cells/100 L/well
Medium: DMEM+10%FCS
Days: 3

Feature3: Grow larger spheroids in same well for long term cultures

Growing larger spheroids needs more media. Slit well plates allows 1.5 times more media volume compared to conventional plates providing more nutrients for larger spheroids.

Reagents and Equipment

PrimeSurface® 96 Slit-well plates.

Cells

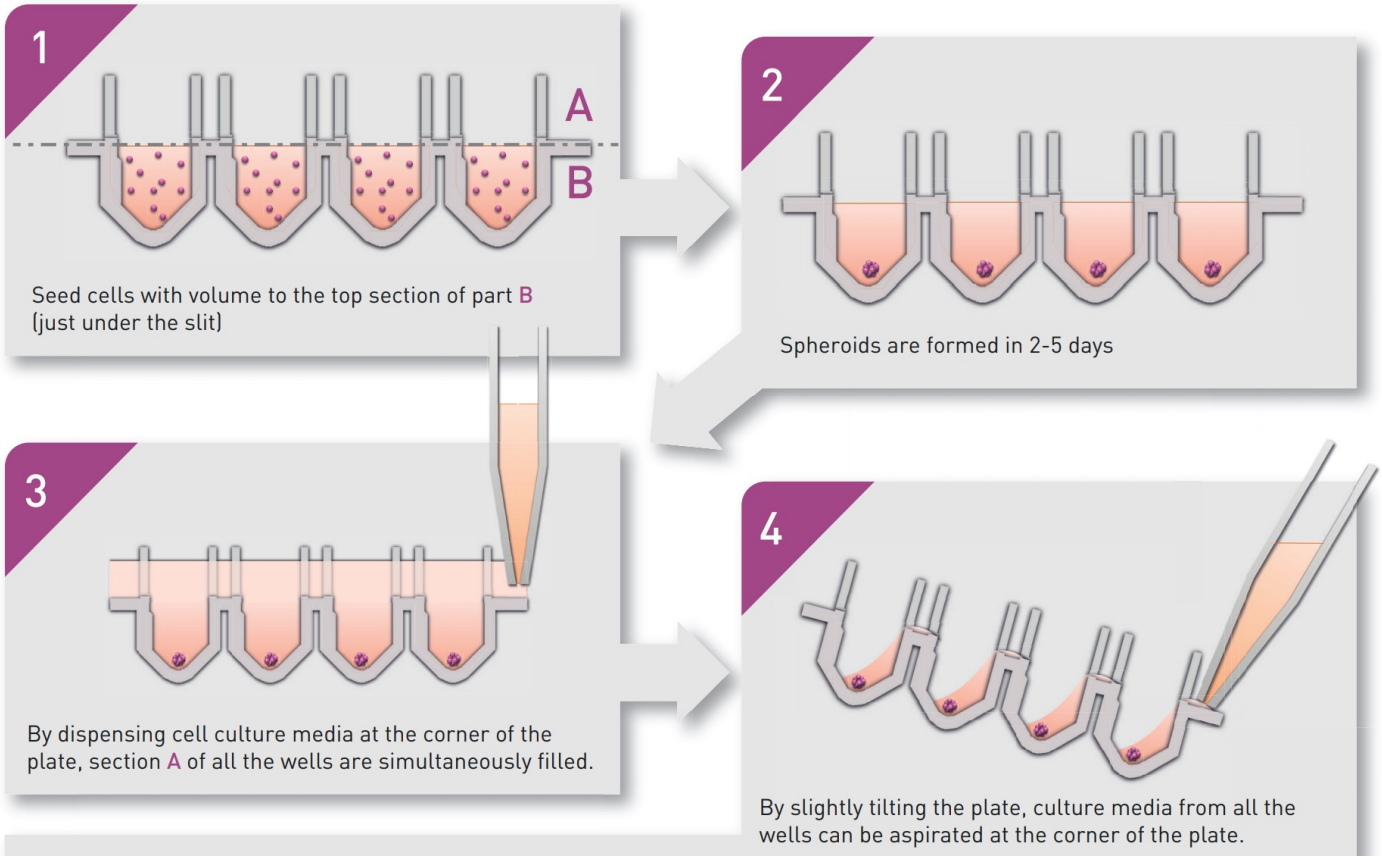
Procedure

Procedures of cell spheroid formation

1. Open the packaging of a new Slit-well Plate under the hood.
2. Seed cells in the plate at a density of 6,000-9,000 cells/100 µL/well.
3. The recommended volume of the above cell suspension is approximately 100 µL.
4. As the maximum capacity of each well is 100 µL, do not exceed 100 µL volume for cell suspension per well.
5. The formed spheroids are apt to slip through the slit or protrude outside the well if the number of seeded cells is insufficient.
6. Dispense the cell suspension at the bottom of each well (section B). (Do not dispense it in section a of the well, i.e. in the slit section).
7. Culture the cells in the incubator.
8. After every 24-48 hrs, check for spheroid formation.
9. Gently and carefully handle the plate during this first 24-48 hrs, as the spheroids being formed in the initial stage are fragile and can break apart.

10. Dispense fresh culture media (20 mL per plate) at the corner of the plate using a 25 mL pipette.
11. The total volume of the dispensed culture media is 30 mL per plate, i.e. an aliquot of 10 mL during cell seeding, plus 20 mL in this step (as stated above).
12. The maximum capacity of the plate is 40 mL/plate.
13. Dispense media slowly (it should take about 20 seconds to dispense 20 mL volume to the plate).
14. Gently shake the plate back and forth in all directions a couple of times to ensure that all the wells have been evenly covered with media.
15. Place the plate in the incubator.

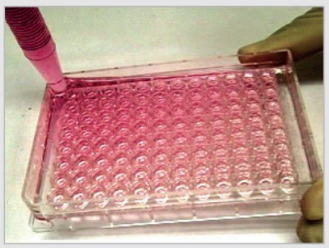
Spheroid formation procedure using PrimeSurface® 96 Slit-well Plate



Procedure for exchanging culture media

1. Take the plate out of the incubator and check the spheroid formation under a microscope before exchanging the culture media.
2. Aspirate the media from a corner of the plate with a 25 mL pipette and dispose of it.
3. Apply the pipette tip to the corner of the plate and gently aspirate the old culture media while slightly tilting the plate.
4. The plate must be tilted in order to properly aspirate the culture media.
5. The recommended aspirating volume is 15 to 20 mL (takes about 20 seconds).
6. Dispense fresh media from the corner of the plate with a 25 mL pipette. This volume should be the same as that of aspiration step (2).
7. Note that the capacity of the slit plate is 40 mL/ plate. It is advised to stay with total volume of 30 mL/plate.
8. Dispense media slowly (takes 20 sec) to avoid any overflow.
9. Gently shake the plate back and forth in all directions a couple of times to ensure that all the wells and corners of the plate have been evenly covered with media.

Place the pipette tip at one corner of the plate



Start aspirating the culture media slowly

Continue to tilt the plate gradually while aspirating the media

Aspirating media in this manner keeps the spheroids in the wells

Time Taken

Long-term cultures

Notes and Comments

1. The product and instruction are subject to change.
2. This product is for research purpose only.
3. PrimeSurface® 96 Slit-well Plate, is designed to form and maintain uniform spheroid for long term cultures.
4. New and innovative design of plate with slits on the upper half of each well.
5. Media aspirating and dispensing is efficiently performed from the corners of the plate while keeping the spheroid formation undisturbed in wells.

References

Not available