Standard Operating Procedure (SOP) for 3D Cell Culture Using HCT116 Cells with AcroCyte R³CE® Type I Plate

1 Introduction

This document describes the standard operating procedure (SOP) for culturing HCT116 cells to form 3D spheroids using the AcroCyte R³CE® Type I Plate. The procedure includes cell collection, culture, spheroid formation, isolation, passaging, and cryopreservation.

2 Materials and Equipment

- HCT116 colorectal cancer cell line
- AcroCyte R³CE® Type I Plate (6-well, 12-well, 24-well, or 96-well)
- Phosphate-buffered saline (PBS)
- Antibiotic-Antimycotic solution (AA)
- Complete culture medium (Dulbecco's Modified Eagle Medium supplied with 10% fetal bovine serum)
- Trypsin-EDTA enzyme solution
- 37°C CO_2 incubator
- Centrifuge
- Cryogenic vials
- Refrigerators at 4°C, -20°C, and -80°C, and liquid nitrogen storage

3 Procedure

3.1 Preparing the R³CE® Plate

Remove the outer packaging of the R³CE® Plate, open the lid, and add PBS containing 5% AA to each well according to the volume listed in the table below. Ensure the PBS fully covers the surface of each well. Let it sit for 30 minutes.

Plate Format	Recommended PBS Volume
6-well	1,000 µL - 2,000 µL
12-well	500 µL - 1,000 µL
24-well	200 µL – 400µL
96-well	100 µL – 200 µL

Gently rinse each well twice with PBS and remove the PBS.

3.2 3D Cell Culture and Spheroid Formation

3.2.1 Cell Seeding

Plate Format	Cell Density (cells/well)
6-well	5.0×10^4 - 2.5×10^5
12-well	2.5×10^4 - 1.5×10^5
24-well	1.0×10^4 - 5.0×10^4
96-well	1.0×10^3 - 1.0×10^4

Replace PBS with culture medium and evenly distributed the seeded cells on the surface of each well.

3.2.2 Culture Conditions

Incubate the seeded R³CE® Plate in a 37°C, 5% CO_2 incubator for 3-7 days.

Change no more than half of the culture medium from the liquid surface every three days, avoid disturbance of the cells.

3.2.3 Spheroid Formation

Cells will form spheroids within 3-14 days in the R³CE® Plate.

Spheroids can be sustained and grown in the R³CE® Plate up to 21 days. Transfer the cultured spheroid or passage the sample before 21 days is highly recommended.

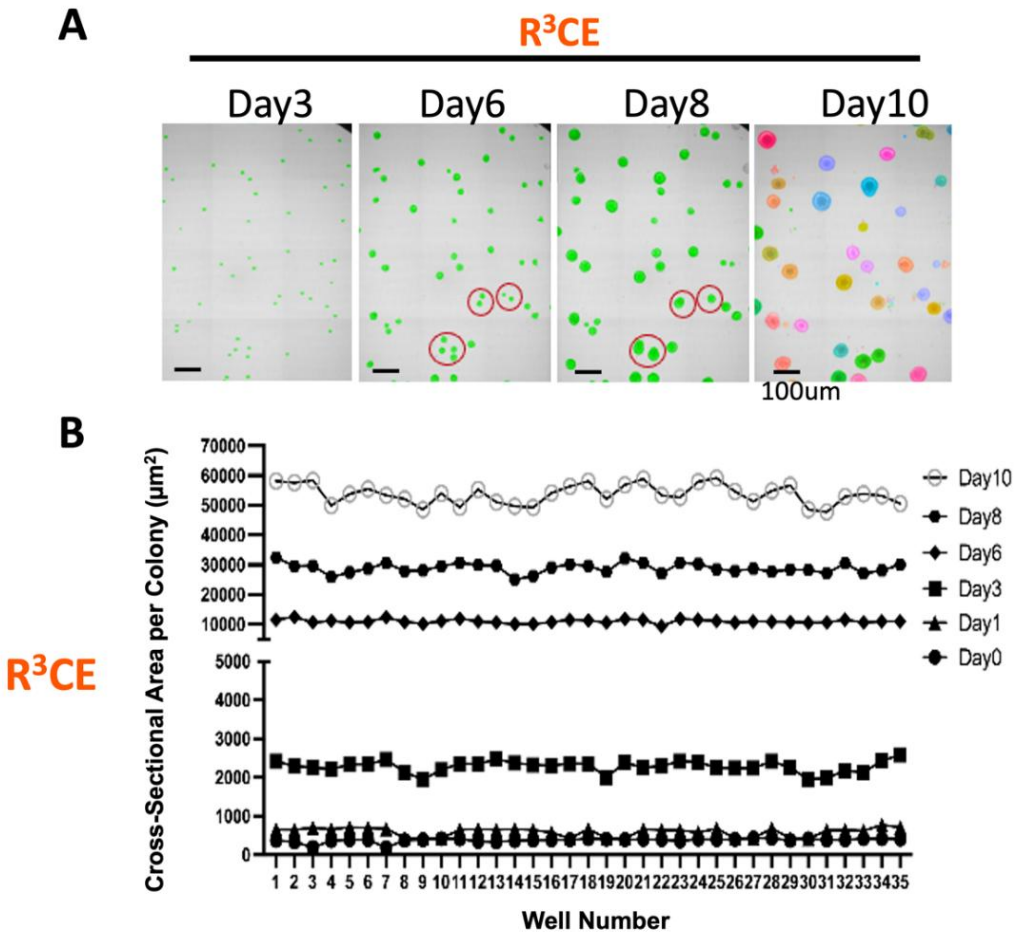

Figure 1 *R^3CE-based cell growth evaluation of the HCT116 colorectal cancer cell line.* ***(A)*** *Image inspection of the HCT116 cells grown on R^3CE platform of day3, day6, day8, and day10. Note that some of the adjacent cells with close distance will finally fused by direct contact and cause decrease in total spheroid count and indicated as red circle. Scale bar: 100 μm.* ***(B)*** *Time-lapsed inspection on average HCT116-derived spheroid area cultured on 96-well R^3CE plate of day0, day1, day3, day6, day8, and day10. The average size of the spheroids represented almost equal in each well during the sample culture period.*

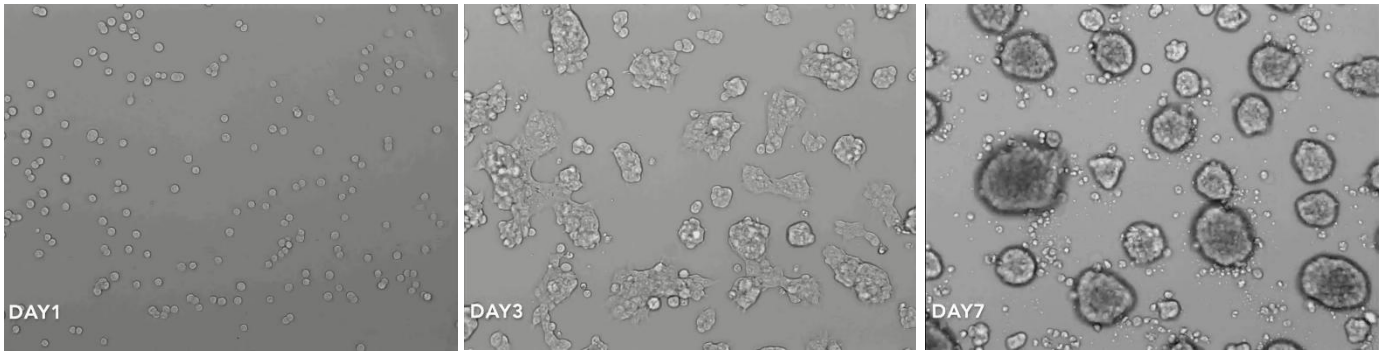

Figure 2 *Photographs of the HCT116 colorectal cancer cell line growth on the R³CE Type I plates: Day 1, Day 3, and Day 7.*

3.3 Spheroid Isolation and Collection

3.3.1 Isolating Spheroids

Gently pipetting the culture medium near the bottom of the wells to detach the spheroids from the R³CE® Plate surface.

Transfer the spheroids containing medium to a collection tube.

Centrifuge at 100 x g for 3 minutes to sediment the spheroids.

Discard the supernatant and collect the sedimented spheroids for further applications.

3.4 Passaging and Reseeding

3.4.1 Passaging Spheroids

Dissociate spheroids by gentle pipetting after 15 minutes incubation with Trypsin-EDTA enzyme solution in a 37°C water bath.

Reseed the dissociated cells and reseed to form new spheroids in an alternative R³CE plate.

3.5 Cryopreservation and Revival

3.5.1 Cryopreservation

Collect spheroids, centrifuge to sediment, and remove the supernatant. Dissociate the spheroids prior to cryopreservation if desired.

Add cryopreservation medium and aliquot the cell suspension into cryogenic vials.

Slowly cool the vials to 4°C overnight, then to -20°C overnight, and finally to -80°C overnight

before storing in liquid nitrogen.

3.5.2 Revival

Rapidly thaw the cryopreserved samples in a 37°C water bath.

Transfer the thawed cells to the R^{3}CE® Plate and add culture medium.

4 Precautions

- ✧ Avoid shaking, sudden movements, direct disturbance or scratching of the R^{3}CE® Plate during culture to prevent cell detachment or damage the surface.
- ✧ Prevent the R^{3}CE® Plate surface from drying after rehydration throughout the culture process.
- ✧ Handle spheroids with care to avoid damaging the R^{3}CE® Plate surface.

5 Storage & Preservation

Preserve the R^{3}CE plate under 4^{o}C refrigerator and prevent from freezing. Avoid the surface from dryout once rehydrate the R^{3}CE platform or direct contact/scratching the coated surface.

6 Technical Support

Contact AcroCyte Therapeutics at *info@acrocyte.com* and provide the product receipt date, unpacking date, and product Lot/SN number for technical support.