Instruction Manual for AcroCyte R³CE® Type I Plate & Flask

Product Name	R³CE® Types I Plate (6/12/24/96-well) R³CE® Types I Flask (25/75/-cm²)
Product Specification	Flat bottom plate
Storage and shelf life	For long-term storage, it is recommended to store in a dark place at a temperature of 4-8℃. The shelf life is nine months from the date of manufacture.
Product Use	Suitable for 3D cell culture

Format	Plate Color	Catalog Number	Dimensions Reference	Growth medium (mL)
6-well	flat bottom clear	T5225006T	Falcon 353046	2.5–3
12-well	flat bottom clear	T5225012T	Falcon 353043 WIDE C011012	1–2
24-well	flat bottom clear	T5225024T	Falcon 353047 WIDE C011024	0.5–1.0
96-well	flat bottom clear	T5225096T	Corning 3599	0.1–0.2
	flat clear bottom white	T5225096W	In Vitro 062096	
	flat clear bottom black	T5225096B	In Vitro 060096	
25-cm²	flat bottom clear	T52250T25	Falcon 353108	5-50
75-cm²	flat bottom clear	T52250T75	Falcon 353136	10-200

A. *Quality Inspections Before Using the R³CE® Plate*

WARNING: DO NOT use the product if any of the following conditions are true:

a. The packaging or product appears damaged
b. The product has exceeded the expiration date
c. Unexpected particles or patterns found on the product surface
d. The product has been used to culture cells passed 21 days

NOTICE: It is normal for ring-shapes or dendritic crystals to appear at the bottom of unopened culture plates and will not affect subsequent cell culture efficacy.

For technical support, please reach out to AcroCyte Therapeutics directly at info@acrocyte.com

B. *Recommended Criteria for Sample Recruitment*

1. Fresh normal/tumor tissue from clinically suspect/diagnosed/relapsed solid tumor cancer patient
2. Surgically resected tissue
3. Core needle biopsied sample

4. Liquid biopsy sample (e.g. blood or other bodily fluids)
5. Viable cell line sample

NOTICE: Non-adherent or suspension cells are not recommended.

NOTICE: The R³CE® Plate is designed for scaffold-free culture. Avoid adding high concentrations of matrix or biomaterials directly onto the surface.

C. 3D Culture-based Organoid/Spheroid Formation

1. Collect cells from desired samples (primary cultured cells, primary cultured biopsies, liquid biopsy samples, or cell lines— adherent cultured cells with attachment phenotype are highly recommended)
2. Remove the outer packaging, open the lid of the R³CE® Plate, and add Phosphate Buffered Saline (PBS) containing 5% Antibiotic-Antimycotic 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
25-cm^2	3,000µL – 5,000µL
75-cm^2	9,000µL – 15,000µL

3. Gently rinse each well twice with PBS. Then, remove the PBS before adding the cell culture medium.
4. Seed desired cells into R³CE® wells.

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
25-cm^2	7.5×10^4 - 7.5×10^5
75-cm^2	2.2×10^5 - 2.2×10^6

5. Incubate the prepared plate in a CO_2 culture chamber at 37 °C with a humidified atmosphere for 3-7 days.
6. Once every three days, gently exchange no more than half of the culture medium.
7. Seeded cells are expected to grow into organoids/spheroids within 3-14 days of cultivation on R³CE®

Plate. Organoid/spheroid cells can be sustained and continue to grow for at least 21 days. It is highly recommended to exchange grown cells onto a new R³CE® Plate before 21 days.

8. Cultured samples can be collected by direct pipetting from the R³CE® Plates. **Be careful** to not scratch the base of the well while pipetting.

WARNING: Avoid shaking or causing sudden movement to the R³CE® plate while culturing. Cell detachment from the base of the well will stop cell growth.

WARNING: Prevent direct contact or from scratching the surface. The damaged surface will cause inconsistent results or failure in sample culture.

D. ***Isolation of 3D Cultured Organoids/Spheroids***

1. Directly pipette the culture medium near the base of the well to detach the organoids/spheroids from the R³CE® Plate surface. **Be careful** to not scratch the base of the well while pipetting.
2. Transfer the organoids/spheroids containing medium into a collection tube.
3. Centrifuge the cells and medium containing collection tube at 100 x g for 3 minutes to create sedimentation of the organoids/spheroids.
4. Discard the supernatant and collect the sedimented organoids/spheroids for further use.

E. ***Passage & Re-seeding of the 3D Cultured Organoids/Spheroids***

1. Collect the cultured organoids/spheroids from the R³CE® Plate.
2. Dissociate the organoid/spheroid cells with Tryp-LE under 37°C water bath for 15 min.
3. Follow **Section C** using the dissociated organoid/spheroid cells for further cultivating more organoids/spheroids.

∗ For technical support, please contact AcroCyte Therapeutics directly (at info@acrocyte.com) and include the product received date, unpackaging date, and product lot number.

∗ For a video tutorial on the Standard Operating Procedure for 3D Cell Culture Using HCT116 Cells with the AcroCyte R3CE® Type I Plate, please refer to the YouTube link: https://youtu.be/BRfxQ-Nt0II or scan the QR code below.