User Guide (UG) for R³CE-based 3D Culture Medium for R³CE®-based 3D Cell Culture

1 Introduction

This document provides the guidance for users handling and operating both the initial, maintenance, or complete (CTC) culture medium during 3D cultivation with the AcroCyte R³CE® Plate. The procedures include medium handling, medium preparation, and medium storage for following R³CE®-based 3D cultivation.

2 Materials and Equipment

- Selected adequate type of R³CE® medium (tissue organoid or CTC) with tissue sub-types (basal, GI, breast, ovarian, or liver) (see **Table 1**).
- 4°C Refrigerator and/or ultralow-temperature refrigerator at -20°C to -80°C.
- (Optional) Antibiotic solution needed for clonal selection.
- **Table 1** The R³CE Medium Kits

Type	Tissue Type	Package Content	Catalog Number
Organoid Medium	Basal	Initial medium, 25 mL Maintenance medium, 50 mL	BSLLA003
	GI (Gastrointestinal Tract)	Initial medium, 25 mL Maintenance medium, 50 mL	GITLA003
	Breast	Initial medium, 25 mL Maintenance medium, 50 mL	BRALA003
	Ovarian	Initial medium, 25 mL Maintenance medium, 50 mL	OVALA003
	Liver (Hepatocellular)	Initial medium, 25 mL Maintenance medium, 50 mL	HEPLA003
CTC Medium	Basal	Complete Medium, 50 mL	CTCLA004
	Breast	Complete Medium, 50 mL	CTFLA004

3 Procedures

3.1 Preparing the cell mixture for initial seeding with R³CE® culture medium

3.1.1 Slowly thaw the initial R³CE® culture medium (for tissue-based samples) or complete medium (for CTC-based samples) below room temperature (preferred thaw on ice) and prevent it from warming up by direct heating.

3.1.2 Gentle pipetting the initial R³CE® culture medium (for tissue-based samples) or complete medium (for CTC-based samples) to homogenize the solution.

3.1.3 Direct mix the cell samples with adequate volume of initial R³CE® culture medium (for tissue-based samples) or complete medium (for CTC-based samples) for initial seeding of the cells on R³CE® culture plate.

3.1.4 The prepared initial R³CE® culture medium (for tissue-based samples) or complete medium (for CTC-based samples) was now ready for further culture applications.

3.1.5 The R³CE® culture medium should be stored under 4°C refrigerator and use within two months.

3.2 Preparing medium exchange with R³CE® culture medium

3.2.1 Slowly thaw the maintenance R³CE® culture medium (for tissue-based samples) or complete medium (for CTC-based samples) below room temperature (preferred thaw on ice) and prevent it from warming up by direct heating.

3.2.2 Gentle pipetting the maintenance R³CE® culture medium (for tissue-based samples) or complete medium (for CTC-based samples) to homogenize the solution.

3.2.3 Carefully remove no more than half of the culture medium from liquid surface within R³CE® culture plate and replenish the culture medium with same volume of the maintenance R³CE® culture medium (for tissue-based samples) or complete medium (for CTC-based samples). Prevent from disturbing the samples cultured in R³CE® culture plate.

3.2.4 The prepared maintenance R³CE® culture medium (for tissue-based samples) or complete medium (for CTC-based samples) was now ready for further culture application.

3.2.5 The R³CE® culture medium should be stored under 4°C refrigerator and use within 3 months.

4 Precautions

- ✧ Avoid direct heating and repeat freeze-&-thaw cycles to prevent degradation of the R^{3}CE® culture medium.
- ✧ Prevent the R^{3}CE® medium from direct radiation, organic solvent or chemical exposure.
- ✧ The medium is for research use only, DO NOT use for clinical diagnosis or therapeutic applications.

5 Storage & Preservation

- ✧ Preserve the R^{3}CE® culture medium under -20~-80^{o}C ultralow-temperature refrigerator. All the initial, maintenance, and complete R^{3}CE® culture medium should be preserved under 4^{o}C refrigerator after thaw and should be used within 3 months.

6 Technical Support

Contact AcroCyte Therapeutics at *info@acrocyte.com* and provide the product receipt date, unpacking date, storage or usage conditions for further technical support.