2021

Generating 3D Cancer Spheroids with Canvas ECM

SOP NUMBER: APP-NOT-003

CELLARIA

Generating 3D Cancer Spheroids Protocol

PURPOSE: This 3D cell culture protocol is used to create crosslinked spheroids of Cellaria cancer cell lines. The 3D environment contains Collagen 0 (Canvas) and cancer-specific laminins, mimicking ECM. Initial spheroid formation occurs in 24 hours, although it takes 72 hours for spheroids to mature. Grown spheroids can be then used for the drug response.

SAFETY: It is the responsibility of the Laboratory Manager and Safety Officer to ensure all laboratory personnel are properly trained in and follow this SOP. Complete RETM culture medium contains cholera toxin. Use 10% bleach to clean up any spills then dispose of materials in biohazardous waste. Proper PPE and aseptic technique are required at all times.

MATERIALS REQUIRED

1. Equipment/ Instruments

1.1. Biological Safety Cabinet (BSC)
1.2. ThermoFisher HERAcell VIOS CO2 Incubator
1.3. Microcentrifuge tube rack
1.4. Balance
1.5. Micropipette
1.6. Pipette Aide

2. Materials/ Reagents

2.1. Canvas Extracellular Matrix Kit
2.2. BioLaminin-511 and Biolaminin-411 (Human Recombinant laminin, LN-511 and LN-411)
2.3. **5%** FBS and **7%** FBS Complete RETM, 37°C
2.4. 96-Well ULA, U-bottom (round bottom) plate
2.5. 15ml Tubes
2.6. 1.5mL Eppendorf tubes
2.7. Micropipette tips
2.8. Serological pipette tips

PROCEDURE

1. Reagents Overview

1.1 Prepare reagents in appropriate media: for wood and stew reagents use **5%** FBS c-RETM and for powder reagents use **7%** FBS, c-RETM.

1.2 Canvas can be used at room temperature and added directly to warm c-RETM. Crosslinker is supplied with the Canvas kit and stored at -20C. Prepare crosslinker solution right before adding to the cell suspension. To prepare Crosslinker Solution, add 1.2mL of c-RETM to the Crosslinker Vial. Immediately freeze excess into single-use aliquots (ex. 200ul) Frozen aliquots of Crosslinker solution must be used within **2 weeks**.

1.3 Use laminin-511 for Wood and Stew and use laminin-411 for Powder. Laminin stock solution is 100ug/mL and seeding concentration is 5ug/mL. Example: for 10ml total volume, use 500ul of 100ug/mL + 9.5mL of cRETM to obtain c-RETM solution containing 5 ug/mL laminin.

Note: The proteins should be handled with care and unnecessary exposure of the protein to ambient temperatures should be avoided. If possible, keep the sample on ice during work.

2. For 3D spheroid cultures: seed 96 well - ULA (Ultra Low Attachment) Plates

2.1. Passage cell line of interest according to each specific cell line protocol for spheroid formation up to the resuspension of cells.

2.2. Obtain cell counts and calculate total number of cells needed for 5000 cells per well.

2.3. Seed half-a-plate of a 96-well plate containing 5,000 cells/well (240,000 Cell total) in suspension with Canvas, laminin and crosslinker.

Note: When cells are almost done centrifuging, take Canvas from the fridge and add appropriate amount to c-RETM to have 25% Canvas in cell suspension. Then, add appropriate amount of laminin to obtain 5ug/mL laminin concentration. Lastly, add crosslinker to the cell suspension.

2.4. Resuspend cell suspension and quickly seed wells with 5000 cells per well in total volume of 100 µL (suspension containing 25% Canvas, 5ug/ml Laminin and 100uL of Crosslinker per 1mL of Canvas). Let cells settle in the bottom of the well (15-25min depending on the cell's size) before placing in the incubator.

Note: Use microscope to see if cells have settled.

2.5. Incubate in 37°C, 5% CO2 for 4-6 hours.

2.6. Gently top with 100ul c-RETM and place back to the incubator until spheroids are at least 200 µm in size.

2.6.1. Spheroid formation usually occurs 24 – 72 hours post seeding.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Cell Lines		
Powder Ovarian Cancer Cell Line	Cellaria Inc.	CB-0111
Stew Pancreatic Cancer Cell Line	Cellaria Inc.	CB-0601
Wood Breast Cancer Cell Line	Cellaria Inc.	CB-0401
Chemicals, Peptides, and Recombinant Proteins		
Renaissance Essential Tumor Medium™	Cellaria Inc.	CM-0001
Canvas™ Extracellular Matrix	Cellaria Inc.	EM-0001
Biolaminin-411 (Human Recombinant laminin)	BioLamina	LN-411
BioLaminin-511(Human Recombinant laminin)	BioLamina	LN-511
Other		
Corning 96 Well Black Clear Round Bottom for ULA Spheroid Microplate with Lid	Corning	Catalog # 4520