

*DOE FOR
CULTIVATION OF
MCF-7 CELLS IN
MATRIGEL*

Experiment 1 of 5

Druhot, Kalyn F

Undergraduate Researcher Fall 2018-Spring 2020

TABLE OF CONTENTS

2	Overview
3	Plate Layout and Timeline
4	List of Materials
5-6	Methods
7-8	Calculations

Overview:

List of Experiments:

- MCF-7 Spheroid Formation
 - Overview: MCF-7 Cells will be plated at varying cell concentrations in mediums with varying composition of Matrigel to investigate which method most reliably produces spheroids.
 - Hypothesis: 4% Matrigel will most reliably produce spheroids.
 - Pre-Lab Calculations: Determine media, Matrigel, collagen, and cell volumes needed for the following dilutions if the total desired volume is 1 mL and the starting stock cell concentration is 1×10^6 cells/mL. Perform dilutions for control groups.
 - Matrigel:
 - ECM 1 : 3% Matrigel
 - ECM 2 : 4% Matrigel
 - ECM 3 : 5% Matrigel

(other groups have looked at adding equal parts collagen 1 to Matrigel and got good results- this might be additional ECM groups to test in the future; for other examples check background literature from Spring 2019)

- Groups:
 - (A) 10,000 cells per well in ECM 1
 - (B) 20,000 cells per well in ECM 1
 - (C) 40,000 cells per well in ECM 1
 - (D) 10,000 cells per well in ECM 2
 - (E) 20,000 cells per well in ECM 2
 - (F) 30,000 cells per well in ECM 2
 - (G) 10,000 cells per well in ECM 3
 - (H) 20,000 cells per well in ECM 3
 - (I) 40,000 cells per well in ECM 3
- Output:
 - Viability via CellTiter-Glo 3D
 - Observe and capture under microscope

Materials:

Plate Precoating

- *Complete MCF-7 growth medium: EMEM + 10% FBS + 1% penicillin/streptomycin + 0.01 mg/mL human recombinant insulin*
- *Matrigel aliquots (3%, 4%, 5%)*
- *Automatic volumetric pipetter*
- *Micropipetter*
- *Pipette tips (COLD)*
- *(1) 96-well black-walled tissue culture-treated microtiter plate (clear bottom)*

Cell Concentrations/Dilutions

- *T-25 mL flask of MCF-7 cells*
- *Aspirating pipettes*
- *20 mL sterile PBS*
- *3 mL frozen trypsin-EDTA (0.25% trypsin/2.21 EDTA)*
- *Complete MCF-7 growth medium: EMEM + 10% FBS + 1% penicillin/streptomycin + 0.01 mg/mL human recombinant insulin*
- *Matrigel aliquots (6%, 8%, 10%)*
- *Automatic volumetric pipetter*
- *10 mL sterile plastic volumetric pipette*
- *5 mL sterile plastic volumetric pipette*
- *15 mL centrifuge tube*
- *Micropipetter*
- *Pipette tips (COLD)*
- *Eppendorf tubes (COLD)*
- *96-well microtiter plate that has been coated during precoating and should be in incubator*

Observation

- *Confocal Microscope*
- *96-well microtiter plate that has been coated during precoating and should be in incubator*
- *CellTiter-Glo 3D Cell Viability Assay*

Methods:

Aliquot Stock Solutions

(only needs to be done once every 4 experiments; see calculations section)

****remember that initial bottle of Matrigel must be thawed by placing it in an ice bath in the fridge the night before.**

SHOULD BE KEPT COLD TO PRESERVE MATRIGEL

1. 4 cuvettes: 2mL (doubled concentration) ECM 1
 - Ex: 6% Matrigel
2. 4 cuvettes: 2mL (doubled concentration) ECM 2
3. 4 cuvettes: 2mL (doubled concentration) ECM 3

Day 0: Preparation

Freeze pipette tips and cuvettes. Thaw Matrigel aliquots by placing them in an ice bath in the fridge.

Day 1: Coat Plates and Seed Cells

Perform Matrigel Dilutions (see calculations section)

Coat Plates

1. Preincubate the 96-well microtiter plates to 37 °C using a plate warmer at 37 °C. Warm media, PBS, and trypsin in 37 °C water bath.
2. Precoat 96-well plates with 60 µL per well of the relevant matrix (according to the plate layout).
3. Place the plates in a humidified incubator at 37 °C for at least 30 min to allow for matrix to set; keep there until cells are ready to add.

Cell Concentrations/Dilutions and Plating

STEPS 1-4 SHOULD BE KEPT COLD TO PRESERVE MATRIGEL

1. Count cells using multisizer
2. Dilute cells to **2x** desired concentration marked on plate layout
3. Add 600uL Matrigel to 600uL cell dilution for mixtures indicated on plate layout
 - ex: 600uL 6% Matrigel + 600uL 20,000 cell concentration
= 1.2mL 3% Matrigel with a 10,000 cell concentration
4. Plate 60uL of cell/Matrigel mixture into each well indicated on plate layout

5. Incubate the plate at in a humidified incubator at 5% CO₂/ 37 °C for 2 h to allow the matrix to set.
6. Add 100 µL of prewarmed complete MCF-7 growth media into the wells.

Day 3: Observation

1. Document Spheroids Using Confocal Microscope

Day 7: Observation & Cell Titer Glo

1. Document Spheroids Using Confocal Microscope

CellTiter-Glo 3D Cell Viability Assay

1. Equilibrate the plate and its contents to room temperature (22–25°C) for approximately 30 minutes.
2. Add a volume of CellTiter-Glo® 3D Reagent equal to the volume of cell culture medium present in each well (e.g., for a 96-well plate, add 100µl of CellTiter-Glo® 3D Reagent to 100µl of medium containing cells).
3. Digest cells with dispase
4. Mix the contents vigorously for 5 minutes to induce cell lysis. Note: Mixing is very important for effective extraction of ATP from 3D microtissues. See the Appendix, Section 4, for more information on mixing.
5. Allow the plate to incubate at room temperature for an additional 25 minutes to stabilize the luminescent signal.
6. Record luminescence.
 1. Notes: Detection instrument settings depend on the manufacturer. Use an integration time of 0.25–1 second per well as a guideline. An uneven luminescent signal within plates can be caused by temperature gradients, uneven seeding of cells or edge effects in multiwell plates.

Calculations:

look up Matrigel spec sheet and find protein concentration for every new bottle and perform calculations as seen below

Current bottle's protein concentration: 10.8mg/mL → this means it starts at 10.8%

All calculations below are for this concentration and for the 3%, 4%, 5% Matrigel Experiment

Initial Aliquot Procedure (performed once every 4 experiments):

Make 4 cuvettes of 2mL for each of these:

- (double conc.) ECM 1 = 6% Matrigel

1. $(10.8)(x) = (6)(2\text{mL})$

In each of the four cuvettes add 1.11mL Matrigel to 0.81mL DMEM

Mix each and refreeze for future use

- (double conc.) ECM 1 = 8% Matrigel

1. $(10.8)(x) = (8)(2\text{mL})$

In each of the four cuvettes add 1.48mL Matrigel to 0.52mL DMEM

Mix each and refreeze for future use

- (double conc.) ECM 1 = 10% Matrigel

1. $(10.8)(x) = (10)(2\text{mL})$

In each of the four cuvettes add 1.85mL Matrigel to 0.15mL DMEM

Mix each and refreeze for future use

(total Matrigel concentration used = 4.44mL)

Dilutions to be done EACH experiment:

Exact volumes of each solution needed total :

- ECM 1: (18 wells) x (60 uL needed for coating)

= 1080uL

- (double conc.) ECM 1: (18 wells) x (30 uL needed for mixing with cell dilution)

= 540uL

- ECM 2: (18 wells) x (60 uL needed for coating)

= 1080uL

- (double conc.) ECM 2: (18 wells) x (30 uL needed for mixing with cell dilution)

= 540uL

- ECM 3: (18 wells) x (60 uL needed for coating)

= 1080uL

- (double conc.) ECM 3: (18 wells) x (30 uL needed for mixing with cell dilution)

= 540uL

Making Matrigel Dilutions:

(this is specifically for protocols making 3%, 4%, 5% Matrigels, but can be used as a template for other concentrations)

1. Remove 1mL solution from 2ml 6% Matrigel Stock solution and place in separate cuvette
2. Add 1mL DMEM to make 2mL 3% Matrigel
(you should now have 1 cuvette with 1mL 6% Matrigel and 1 cuvette with 2mL 3%matrigel)
3. Repeat this process for the 8% and 10% stock solutions

This will give you:

- 1 cuvette with 1mL 6% Matrigel
- 1 cuvette with 2mL 3%matrigel
- 1 cuvette with 1mL 8% Matrigel
- 1 cuvette with 2mL 4%matrigel
- 1 cuvette with 1mL 10% Matrigel
- 1 cuvette with 2mL 5%matrigel