FOOD SAFETY LABORATORY
CORNELL UNIVERSITY

Maintenance of Caco-2 Cells

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TABLE OF CONTENTS

SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth guidelines for maintaining Caco-2 human intestinal epithelial cells for experimental purposes.

1.2 Scope

This SOP applies to the Food Safety Lab, including the Laboratory for Food Microbiology and Pathogenesis of Foodborne Diseases.

1.3 Definitions

Split Ratio: The split ratio defines the ratio with which cells are diluted when they are passages in to a new flask. For example, if ½ of the cells in one confluent T75 are seeded into a new T75 flask, then the cells were split at a 1:2 ratio.

1.4 Safety

Caco-2 cells represent a human derived cell line and *L. monocytogenes* is a BSL-2 pathogen. Appropriate protective measures need to be taken when working with both. All waste from these experiments needs to be treated as BSL-2 biohazardous waste.

SECTION 2 MATERIALS

- Caco-2 media (MEM Alpha 1X) with 20% FBS (Gibco 12561-056 500mL)
- PBS. Lab stock in media room pH 7.4
- 0.1% Trypsin-EDTA
- Corning sterile tissue culture flasks
- Sterile 2, 5, 10, 25 mL stereological pipettes.
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SECTION 3 PROCEDURES

3.1 Routine maintenance of Caco-2 cells in T75 flask

A typical tissue culture user should maintain Caco-2 cells in a T75 flask to have a continuous supply of cells for invasion assays and other experiments. For routine maintenance of Caco-2, cells should be split at a subcultivation ratio of 1:4 to 1:6 (ATCC recommendation). Full confluency should be reached approximately every 4 to 6 days respectively. In general, flasks can be seeded at lower split ratios if cells are needed faster for additional experiments. If cells are simply being maintained, a 1:6 split ratio is more appropriate.

Guidelines for splitting ratios:

Split Ratio	Cell Suspension Used (mL)
1:3	3.0
1:4	2.2
1:5	1.8
1:6	1.5

3.2 Splitting Caco-2 cells

1. Check cells daily under inverted microscope to check for indications of contamination (visible growth of contamination, cloudy or discolored media, etc.) and to monitor growth rate of cells (if cells are not growing at proper rate, consult a senior technician or grad student with tissue culture expertise).
2. When cells are 80-100% confluent, prepare all necessary reagents and materials in the hood.
3. Remove media and wash monolayer with 10 mL sterile PBS.
4. Add 2 mL 0.1% Trypsin-EDTA and incubate the flask in the 37C incubator for 2 minutes.
5. After 2 minutes, check cells under inverted microscope for detachment. If some small clumps still remain attached to flask, rap flask on counter to jar and dislodge cells.
*This step should be done as quickly as possible, because long exposure to trypsin will permanently damage the cells.
6. Add 7 mL of pre-warmed media and "wash" flask to remove any remaining cells from the flask. Pipette up and down vigorously to break apart cells that clump to one another. Check cells again under inverted microscope. Cells should not be clumped or balled together and should be floating freely.
7. Split cells into a new flask at desired split ratio above; bringing up total volume in a new T75 to 20.0 mL.

SECTION 4 REPORTING and LABELING

Label each tissue culture flask with the following:
Caco-2
Split Date/ Initials
Split Ratio
Passage Number

Record all cell maintain in official laboratory notebook.

SECTION 5 TROUBLESHOOTING

Previous Trypsin-EDTA solution was a 0.25% solution (without glucose) which proved to be too harsh for Caco-2 cells; potentially stripping away the cells surface proteins and permanently damaging them. Cells would not recover and proliferation slowed to a halt. Additionally, cells

would not reattach, many would never reattach and float, and monolayer was extremely delicate as a result. Cells appeared much healthier with new trypsin formulation. Decreasing percentage of trypsin solution did not affect detachment time during passaging and cell maintenance.

Cell vacuolization is not normal. Monolayer detachment is not normal. Decreased growth is not normal. If any of these conditions arise, record observations and consult a laboratory technician.

SECTION 6 REFERENCES

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Freshney, R. I. 2000. Culture of Animal Cells. 4th ed. Wiley-Liss New York.

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