



FOOD SAFETY LAB / MILK QUALITY  
IMPROVEMENT PROGRAM

*Standard Operating Procedure*



Title: **Maintenance of Caco-2 Cells**

SOP #: **see Wiki**

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Author: **Emily Wright**

Approved by: **Martin Wiedmann**

## **Maintenance of Caco-2 Cells**

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## 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 into a new flask. For example, if  $\frac{1}{2}$  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.
- 0.1% Trypsin-EDTA (This medium is purchased (Gibco 25300-054))
- Corning sterile tissue culture flasks
- Sterile 2, 5, 10, 25 mL seriological pipettes.



## **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 sub-cultivation 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 sooner 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 biosafety cabinet.
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 37°C 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 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 with MEM  $\alpha$  with 20% FBS.

### **3.3 Reporting and Labeling**

Label each tissue culture flask with the following:

- Caco-2
- Split Date/ Initials
- Split Ratio
- Passage Number



## SECTION 4                      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 5

## REFERENCES

*Martin B. M. 1994. Tissue Culture Techniques: An Introduction. Boston: Birkhauser.*

*Freshney, R. I. 2000. Culture of Animal Cells. 4<sup>th</sup> ed. Wiley-Liss New York.*

*Powers J, Cooper L, Nelson C, Everts M. 2008. Trypsin Solution One Liter. Animal Health Diagnostic Center Virology Laboratory.*



**SECTION 6**

**METHOD VERSION & CHANGES**

<b>VERSION</b>	<b>DATE</b>	<b>EDITOR</b>	<b>COMMENTS</b>
Version 1	11/22/2010	Emily Wright	Original SOP
Version 2	04/24/2020	Maureen Gunderson & Rachel Cheng	- Updated SOP to new standard lab template. - Added product number for Trypsin EDTA