

## **Supplemental Material A.**

### **Cell Culture Experiment**

#### **1. Preparing 10% tissue culture growth media and 2% maintenance media**

##### 10% tissue culture growth media (A549 or BGM)

500ml Minimum Essential Media (MEM)\*  
50ml Fetal Bovine Serum (FBS)  
6 ml Antimycotics  
6 ml Kanamycin  
6 ml Sodium pyruvate  
6 ml NEAA  
10 ml HEPES buffer

##### 2% tissue culture maintenance media (A549)

500ml Minimum Essential Media (MEM)\*  
10ml Fetal Bovine Serum (FBS)  
6 ml Antimycotics  
6 ml Kanamycin  
6 ml Sodium pyruvate  
6 ml NEAA  
10 ml HEPES buffer

#### **2. Cell Passage Procedure**

1. Examine cell line for confluence of at least 80-90% confluence. If not, following cell passage procedures can NOT be carried out.
2. Turn on ultraviolet light in biosafety cabinet for 30 minutes. Then spray with 10% bleach solution (bleach solution should be made fresh before hand) and allow to sit for a 10 minute contact time. Wipe up bleach with paper towel, then spray hood down with 70% ethanol. Wipe up the ethanol, Spray cabinet again with ethanol and allow the ethanol to completely evaporate.
3. Warm tris buffered saline (TBS), 10% growth medium, and trypsin in 37°C water bath. (Trypsin should be warmed briefly right before use. Holding trypsin at high temperatures for long periods will inactivate the enzyme.) After this is done, wipe all bottles with 70% ethanol.
4. Empty old media from flask into a nalgene bottle filled with at 5% house hold

bleach. Rinse flask twice with 10 – 30 ml TBS depending on the size of the flask. Rotate the flask to rinse the inner walls and discard the solution to the nalgene bottle with bleach. All Flasks should be flamed each time they are opened or closed.

5. Add appropriate amount (see below) of warmed PBS - EDTA and trypsin. Incubate the flask at 37°C for 7 minutes. Incubate longer only if needed.

**Table A-1.** Volume of Trypsin and PBS with EDTA used for different sizes of flasks

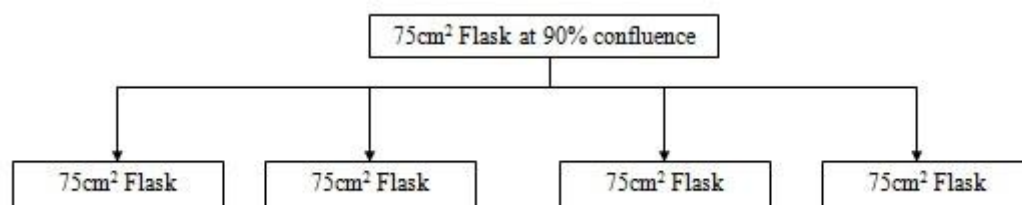
Flask Size (cm <sup>2</sup> )	PBS with EDTA (ml)	Trypsin (ml)
25	2	1
75	6	3
150	12	6

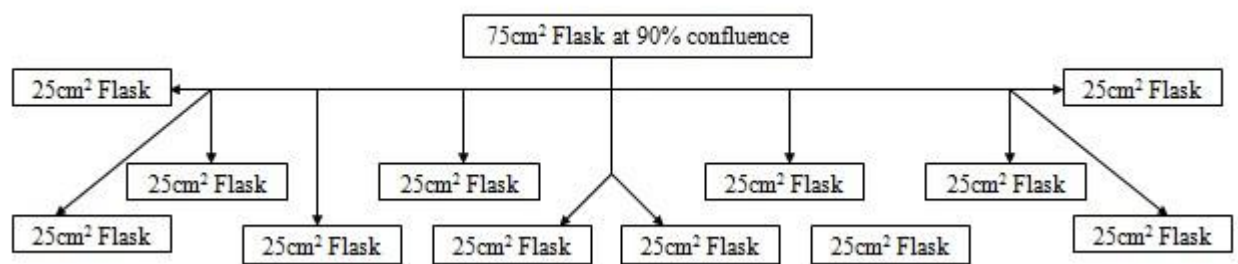
6. Remove flask from incubator and tap the flask gently on the bottom of the flask. Check cell detachment with microscope.

7. Transfer the entire content in the flask to a 50ml centrifuge tube. Concentrate the cells by centrifugation (400× g, 4 min), and then discard the supernatant into bleach (Be very careful and not discard the cells into bleach, it is ok to leave a little trypsin solution in the tube).

Optional: Rinse the flask twice the PBS and discard into bleach during centrifuge. Flasks can be reused up to four times before discarding them for new ones.

8. Resuspend the cells with growth medium. The amount of growth medium depends on the split ratio and size of flasks. For example, with 1:4 split, cells from a 75 cm<sup>2</sup> flask can be distributed into four 75cm<sup>2</sup> flasks or twelve 25cm<sup>2</sup> flasks (one 75cm<sup>2</sup> equivalent to three 25cm<sup>2</sup> flasks), as shown below. Then 24ml growth medium may be used to resuspend the cells. Add 6ml of the cell suspension (solution with cell and grow medium) to each 75cm<sup>2</sup> flask or add 2ml of the cell suspension to each 25cm<sup>2</sup> flask. Be sure to break up clumps by vortexing cell suspension and vigorously pipetting suspension up and down in the tube.





**Figure A-1.** Aliquoid of cell suspension from 75 cm<sup>2</sup> flask to 75 cm<sup>2</sup> and 25 cm<sup>2</sup> flask with split ratio of 1:4

9. Add appropriate amount of growth to flasks and make the total medium volume of different sizes of flasks as shown in Table A-1. For example, if 2ml of cell/grow medium solution has been added into a 25cm<sup>2</sup> flask, then 7ml more growth medium needs to be added in the flask to make the total medium volume of 9ml.

**Table A-2.** Volume of growth medium for different sizes of flasks

Flask Size (cm <sup>2</sup> )	Total Medium Volume
25	9
75	25
150	50

10. Rock the flasks and keep them in the 37°C incubator. Check if the cells landed on the bottom of the flask in the next day. For 1:4 split, generally it takes 2 days to reach >80% confluency.

### 3. Cell Culture Assay with Environmental Samples

1. Check all flasks with cell line for at least 90% confluence. If not, following cell culture assay procedures can NOT be carried out.
2. Thaw samples in room temperature or 37°C water bath. If water bath is used, remove samples from water bath immediately after they are completely thawed. All bottles and samples should be wiped up with 70% ethanol afterward.
3. Disinfect the bench top where the assay is to be performed with 10% bleach (allowing for a 10 minute contact time), followed with 70% ethanol. Light the Bunsen burner in the work area.
4. Pull out fresh flasks to be assayed on and stand them up in a line on the bench. Label each flask with sample ID, flask number, and date. If the samples need to be diluted with MEM before adding cells, do so now.

5. Discard medium from fresh cell culture flasks into bleach.
6. Mix Inoculate each flask with the desired sample with appropriate volume (1 ml of inoculant for a 25cm<sup>2</sup> flasks and 3 ml for a 75cm<sup>2</sup> flasks). Inoculum must cover cell layer. If sample size is low, add 0.5 - 1ml TBS or MEM to increase the volume.
7. Rock the flasks and incubate them in 37 °C incubator for 60 minutes. Rock flasks every 15 minutes during incubation (prevent drying of cells).
8. Discard the samples (inoculum) into bleach and add appropriate volume of maintenance medium (2%) to each of the flasks, as shown in the table below.

**Table A-3.** Volume of maintenance medium for different sizes of flasks

Flask Size (cm <sup>2</sup> )	Total Medium Volume
25	8
75	22
150	44

9. Keep all flasks in the 37 °C incubator. Check for CPE daily. All flasks positive for CPE should be kept in -80 °C freezer. After 14 days, samples with no CPE occurred can be considered as non-infectious samples.

### **Changing Medium for Cell Culture Assay**

1. Warm maintenance media (2%) in water bath.
2. Check all flasks in assay for CPE. (If a flask showing signs of CPE, do NOT change media, but keep it in the -80 °C freezer for confirmation)
3. Clean counter with 70% ethanol and turn on Bunsen burner. All bottles of warmed medium should be wiped down with ethanol before starting procedure.
4. Lightly flame the mouth of the flask and discard old medium into bleach. Flame mouth and set cap on flask.
5. Add appropriate amount ((8 ml for 25cm<sup>2</sup> flasks and 20 ml for 75cm<sup>2</sup> flasks)) of maintenance medium (2%) into flask, flame and close the flask. Proceed to next flask.
6. Place all flasks back in 37 °C incubator.