# Culturing, Maintaining, & Seeding Detroit 562 Cells

**Created by:** Alejandra Lagos **Date:** April 13th, 2016
Bowdish Lab, McMaster University
Hamilton, ON, Canada, L8S 4L8
www.bowdish.ca

## Background:

This is a human-derived pharyngeal carcinoma cell line. This particular cell line is originally derived from a metastatic site (pleural effusion) of pharyngeal tissue from a Caucasian adult female. They maintain many properties from pharyngeal epithelial cells, including the production of antimicrobial peptides. They also express certain functional TLRs (e.g. TLR-3) and the vitamin D receptor[1]. They grow well when they are at least 40-50% confluent, and tend to die very quickly if the cultured population is sparse.

## Notes:

**Splitting:** These cells grow very well when they are confluent, and they tend to grow on top of each other once 100% confluency has been reached (they do not die right away). Due to this, splitting these cells takes a bit longer as compared to other cell lines. They will need a minimum incubation time period of 20-25 minutes with trypsin at 37°C and 5% $CO_2$. Alternatively, they can be left to trypsinize at room temperature (in the biohood) and they will still be healthy, but this method will require an approximate time period of 30-35 minutes to completely lift the cells from the flask.

## Equipment & Materials:

**Equipment:**

- Level 2 biohood
- 37°C, 5% $CO_2$ incubator
- Centrifuge
- Haemocytometer
- Tissue culture treated flasks (75 $cm^2$, 250 mL (T75) or 150 $cm^2$, 600 mL (T150))
- 10 & 50 mL falcon tubes
- 2 mL vials (for freezer stocks)
- Pipette gun & tips

**Materials:**

- DMEM + 10% FBS & L-glutamine (Pen/Strep is optional but suggested to prevent contamination)
- 1x PBS (heated to 37°C)
- Trypsin

- Trypan blue
- DMSO
- FBS

## Protocol

**Thawing & Culturing Cells:**

1. Warm DMEM in water bath to 37°C
2. Take 1 freezer stock (vial) and thaw rapidly in 37°C water bath by swirling
3. Pipette the contents of the vial (which contains cells in 1 mL FBS with 10% DMSO) to a 10 mL falcon tube
4. Spin tube in centrifuge at 15,000 rpm for 5 minutes (this removes the DMSO)
5. Resuspend cells in 1 mL of DMEM
6. Add DMEM to a T75 flask (add 19 mL) or a T150 flask (add 29 mL)
7. Add the 1 mL of cell suspension to its respective flask
8. Incubate at 37°C, 5% $CO_2$ until they are 70-80% confluent, then split into a new flask

**Splitting Cells:**

1. Once cells are 70-80% confluent, they will be ready to split
2. Warm DMEM and 1x PBS to 37°C
3. Remove old DMEM from flask with a pipette
4. Wash once with PBS (approximately 10 mL for a T75 flask or 20 mL for a T150 flask)
5. Add trypsin to the flask (same volumes as PBS)
6. Incubate flask with trypsin in 37°C, 5% CO2 incubator for ~20-25 minutes (check under the microscope to see if cells have been lifted once the 15 minute mark has been achieved because the time for lifting depends on the confluency of the flask)
7. Alternatively, the cells can be incubated with trypsin at room temperature in the biohood for ~30-35 minutes (check constantly under the microscope to see if the cells have been lifted once the 25 minute-mark has been achieved) **Note:** you may gently bang the flask to help the cells be lifted faster
8. Add DMEM to the flask to stop the reaction (15 mL to a T75 flask or 25 mL to a T150 flask)
9. Pipette the cell suspension into a 50 mL falcon tube
10. Centrifuge at 15,000 rpm
11. Resuspend the cell pellet in 10 mL of fresh and warm DMEM
12. Pipette the cell suspension (usually 1:2 or 1:5 dilution) into a new T75 flask or T150 flask
13. Add DMEM to the flask and incubate the flask at 37°C, 5% $CO_2$ until 70-80% confluency is reached **Note:** usually, you would want to aim for final volumes of ~20 mL in a T75 flask or ~30 mL in a T150 flask

**Freezing Cells**

1. Perform steps 1-10 from the **Splitting Cells** procedure
2. Resuspend cell pellet in 1 mL of FBS
3. Pipette 10 μL of trypan blue into a 1.5 mL RNase-free microcentrifuge tube
4. Pipette 10 μL of the cell suspension into the 1.5 mL tube

5. Pipette 10 μL of the cell suspension from the 1.5 mL tube into a haemocytometer, count the cells, and decide how many freezer stocks will be made **Note:** aim for 2-3 million cells per vial
6. Add the appropriate amount of freezing media (FBS with 20% DMSO) and FBS to the cell suspension **Note:** DMSO will dilute to 10% when freezing media is mixed with an equal volume of the cell suspension in FBS

**e.g.** If you have 8 million cells, that will make 3 vials. Since you have these cells resuspended in 1 mL of FBS (from step 2), then add 0.5 mL of FBS to this cell suspension. Then, make a freezing media that has a total volume of 1.5 mL (consists of 1.2 mL of FBS + 300 μL of DMSO) so that when you pipette both the cell suspension and the freezing media together, the final volume will be 3 mL of FBS with 10% DMSO.

7. Aliquot 1 mL of FBS with 10% DMSO into each vial
8. Store the vials in the -80°C freezer in a freezing bomb overnight (this will allow for a slower cooling rate and is a requirement for freezing Detroit 562 cells)
9. The next day, transfer the vials to liquid nitrogen in the vapour phase for long-term storage

**Seeding Cells**

1. Perform steps 1-11 from the **Splitting Cells** procedure
2. Pipette 10 μL of trypan blue to a 1.5 mL RNase-free microcentrifuge tube
3. Pipette 10 μL of the cell suspension to the 1.5 mL tube
4. Pipette 10 μL of the cell suspension from the 1.5 mL tube into a haemocytometer and count the cells
5. Seed the desired number of cells into a tissue culture treated plate, such that the well will be 70% confluent **Note:** If using a 12-well plate with a flat bottom and a low evaporation lid, the ideal number of cells is 450,000 per well
6. Fill the well up to 1 mL with DMEM
7. Incubate the plate for 24 hours in the incubator at 37°C and 5% $CO_2$ (this will allow the cells to adhere to the bottom of the well)
8. After 24 hours, perform the required experiment (**e.g.** stimulation of cells)

## Links & References:


1. Matijevic, T., Marjanovic, M. & Pavelic, J. Functionally active toll-like receptor 3 on human primary and metastatic cancer cells. *Scand. J. Immunol.* **70,** 18–24 (2009).

2. http://www.atcc.org/products/all/CCL-138.aspx