# hOTR-CHO CELL CRYOSTOCKS

**MATERIALS**

Fetal Bovine Serum
Trypsin 0.25%
Pen-Strep Solution
Cryostock Tube

**NOTES**

1. All cell culture procedures should be done in a laminar flow hood that has been sterilized with UV light for 10 minutes and has been wiped down with 70% ethanol.
2. Split cells or cryopreserve when they are ~ 75% confluent (in logarithmic growth).

**PROCEDURE**

1. Complete media

| | |
|---|---|
| 500 mL | Hams F12 |
| 50 mL | FBS |
| 7.5 mL | HEPES buffer |
| 5 mL | Pen-Strep |
| 200mg | G418 |

   Dissolve G418 in 5 mL of media. Sterile filter back into media. Aliquot in 50 mL conical tubes.
2. Freezing solution
   9.5 mL Complete Media
   0.5 mL DMSO
3. Warm complete media solution to 37 ºC in waterbath.
4. Pre-warm trypsin solution and fresh media at 37ºC.
5. Carefully remove spent media leaving cells adhered to the flask.
6. Add 5 mL trypsin solution to culture flask and gently distribute over surface of cells. Let stand 5 minutes.
7. Loosen cells by gently rapping on the side of the culture flask.
8. Transfer cells in trypsin solution to a 50 mL centrifuge tube and add 10 mL fresh media.
9. Pellet cells by centrifugation at 1500 rpm for 3 minutes.
10. Decant media and trypsin solution.
11. Resuspend cells in 10 mL freezing solution by gently mixing.
12. Aliquot 1 mL of suspended cells into 1.5 mL cryostock tubes prelabeled with the cell line, split, date, and your initials.
13. Place the aliquots into the freezing apparatus in -80ºC and incubate overnight.
14. The next day, move to liquid nitrogen tank for long term storage.

# hOTR-CHO CELL CULTURE FROM CRYOSTOCK

**MATERIALS**

Hams F12 media
Fetal Bovine Serum
Pen-Strep Solution
Cryostock culture
Cell Culture Flask

**PROCEDURE**

1. Complete media

| | |
|---|---|
| 500 mL | Hams F12 |
| 50 mL | FBS |
| 7.5 mL | HEPES buffer |
| 5 mL | Pen-Strep |
| 200mg | G418 |

   Dissolve G418 in 5 mL of media. Sterile filter back into media. Aliquot in 50 mL conical tubes.
2. Warm complete media solution to 37 ºC in waterbath.
3. Label the flask with the culture, date, and generation of splitting.
4. Pipette 20 ml of complete media solution into 75cm$^2$ cell culture flask(s).
5. Thaw the cryostock tube quickly by mixing with warmed media. When the culture is thawed, pipette it into the flask(s). Gently mix the solution.
6. Incubate the culture at 37ºC in the $CO_2$ incubator for at least 4 hours or overnight.
7. Place a 20 ml aliquot of complete in the waterbath.
8. When the new media is warmed, decant off the old media, and transfer the 20 ml of new media into the culture.
9. Exchange media as necessary.

# hOTR-CHO CELL SPLITTING/PLATING

**MATERIALS**

Hams F12 media
Fetal Bovine Serum
Pen-Strep Solution
Cryostock culture
Cell Culture Flask

**PROCEDURE**

1. Complete media

| | |
|---|---|
| 500 mL | Hams F12 |
| 50 mL | FBS |
| 7.5 mL | HEPES buffer |
| 5 mL | Pen-Strep |
| 200mg | G418 |

   Dissolve G418 in 5 mL of media. Sterile filter back into media. Aliquot in 50 mL conical tubes.
2. Warm complete media to 37 ºC in waterbath.
3. Pre-warm trypsin solution and fresh media at 37ºC.
4. Carefully remove spent media leaving cells adhered to the flask.
5. Add 5 mL trypsin solution to culture flask and gently distribute over surface of cells. Let stand 5 minutes.
6. Loosen cells by gently rapping on the side of the culture flask.
7. Transfer cells in trypsin solution to a 50 mL centrifuge tube and add 10 mL fresh media.
8. Pellet cells by centrifugation at 1500 rpm for 3 minutes.
9. Decant media and trypsin solution.
10. Resuspend cells in 5 mL fresh media by gently mixing.
11. In a new tissue culture flask, combine 19.5 mL fresh media and 0.5 mL of cell suspension.
12. Label the flask with the culture, date, and generation of splitting.
13. Return the culture to the incubator.
14. Add 50 uL cell solution to 450 uL media and count the cells in all four quadrants and take an average. Divide by 40.
    a. Plate at 0.5 million cells/mL in 96 well plate.
    b. Plate at 25,000 cells/mL in 24 well plate.