Title: Split Subcculturing of CHO-K1 Cell Line into a 24-well Plate

Approvals:

Preparer:_W. H. Woodruff_____ Date _20 June 2014_____
Reviewer:_____ Date _____
Reviewer:_____ Date_____

1. Purpose:.This SOP will provide the necessary instructions to subculture the CHO-K1 cell line.

2. Scope: These instructions will enable any qualified technicians to subculture any adherent cell line that can be dissociated with the trypsin enzyme.

3. Responsibilities:
   - 3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
   - 3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. References:
   - 4.1. SOP: ID# REA-001 Preparation of RPMI Stock Reagent
   - 4.2. SOP ID# REA-002 Preparation of 1% and 0.25% Trypsin Stock Reagents
   - 4.3. SOP ID# REA-003 Preparation of RPMI 1640 Growth Media
   - 4.4. SOP ID# REA-004 Preparation of Dulbecco's Phosphate Buffered Saline
   - 4.5. SOP ID# REA-005 Preparation of Penicillin and Streptomycin Reagent for Cell Culture
   - 4.6. SOP ID# BTC-010 Preparation of the Laminar Flow Safety Cabinet, Class II, for Aseptic Cell Culture Protocols
   - 4.7. SOP ID# BTC-011 Set Up and Operation of the Olympus Inverted Microscope for Cell Viewing

5. Definitions:
   - 5.1. Subculture: the process of expanding growing cells into new cultures vessels by dissociating and distributing the cells.
   - 5.2. Monolayer:
6. Precautions:
   - 6.1. All technicians must wear the appropriate PPE for aseptic cell culture work.
   - 6.2. All equipment, reagents and cells must be handled aseptically.
   - 6.3. Trypsin administered at a high concentration or for too long in contact with the cells will result in a lowered viability.

7. Materials:
   7.1. Sterile 2 ml, 5 ml and 10 ml pipettes
   7.2. Drummond pipette aid
   7.3. One sterile 24-well tissue culture plate
   7.4. 400 ml beaker for waste and discard liquids
   7.5. 100 ml RPMI 1640 growth media (see SOP)
   7.6. 100 ml Dulbecco's Phosphate Buffered Saline (D-PBS) (see SOP)
   7.7. 5 ml 0.25% trypsin (see SOP)
   7.8. 85%+ confluent CHO-K1 culture

8. Procedures:
   8.1 General
      8.1.1. Gather and inventory all the required materials.
      8.1.2. Prepare the Laminar Flow Safety Cabinet, for aseptic culture work (see SOP)
      8.1.3. Disinfect with 75% ethanol (spray and wipe) and place all required materials into the Laminar Flow Safety Cabinet

   8.2. Dissociating the cells from the flask surface
      8.2.1. Obtain a CHO-K1 culture from the incubator and examine using the inverted microscope. Check for good morphology, no excess turbidity (sign of contamination), % confluency and degree of floating, rounded cells. A small amount is an indication of cells dividing; large numbers are often an indicator of cell death.

      8.2.2. Aseptically remove the culture media in the flask and discard in the waste beaker.
         NOTE: gently "sloshing" the flask before removing the spent culture media will help increase the viability of your culturing by ensuring the dead cells are loose and floating for easier removal.

      8.2.3. Rinse the cell monolayer with 5 ml of D-PBS.
         8.2.3.1. Add 5 ml of D-PBS to the flask
         8.2.3.2. "Swirl" the flask to ensure rinsing in all corners and edges
         8.2.3.3. Remove the D-PBS to the waste beaker
         NOTE: the purpose of this step is to remove all residual Fetal Bovine Serum (FBS) which has an inhibitory effect on the trypsin

      8.2.4. Repeat the rinse step (8.2.3.) once

      8.2.5. Add 1 ml of 0.25% trypsin to the now empty T-25 flask with the CHO-K1 cells
         8.2.5.1. Carefully swirl to ensure the entire cell layer is covered with trypsin

8.2.6. Place the flask into the 37^{o}C incubator

8.2.6.1. Check the cells for dissociation after 4 – 5 minutes by looking at them under the inverted microscope. Dissociated cells will be rounded and many will be floating. If the majority of the cells are not yet rounded return the flask to the incubator and check again in 3 minutes. Continue this process until ~80% of the cells are rounded and/or floating.

8.2.7. When ready, gently rap the flask against the palm of your hand to dislodge the rounded cells from the surface of the flask into a single cell suspension.

8.2.8. Quickly add ~5.5 ml of RPMI 1640 Growth Media to the 1 ml of trypsin/Cell suspension to total ~6.5 ml of cell suspension.

NOTE: the FBS in the growth media will inactivate the protease activity of the trypsin

8.2.9. Using a 5 ml pipette, triturate the cells by rapidly drawing up and expelling the cells while loosely holding the pipette a corner of the flask. The goal is to achieve a single cell suspension without generating a lot of foam.

TIP: Do not draw up nor expel all of the liquid. Leaving ~ 0.5 ml in the flask or in the pipette will greatly reduce the amount of foam generated.

TIP: At this point, check under the inverted microscope should reveal an ~95% single cell suspension. Repeat trituration if necessary.

8.3. Distribution of cells to fresh flasks

8.3.1. Preparing the 24-well plate

8.3.1.1. Using a Sharpie, label the left side of the plate with the cell line, the growth media used, the date and your initials

8.3.1.2. Draw an “X” across the lid over the12 wells on the right side of the plate

8.3.1.2. Add 0.5 ml of RPMI 1640 growth media to each of the 12 wells on the left side of the plate, 3 columns x 4 rows

8.3.2. For an ~1:12 split of the cells, start by making sure the cells in the flask are well suspended by gently shaking the flask. Transfer 0.5 ml of the cell suspension to each of the 12 wells with the 0.5 ml of growth media.

8.3.3. Discard all the remaining cells/liquid from the original (mother) flask, add 6 ml of fresh RPMI 1640 growth media back into the flask.

8.3.4. Return the plate and the flask to the incubator.

8.3.5. Check all cultures for contamination after 24 hours.

10. History:

Name	Date	Amendment
W.H.Woodruff	20 June, 2014	Initial release