OHAUS®

Cell Culture Passaging Protocol

Developed by OHAUS Corporation

Preparation of Cell Growth Media

1. Thaw all reagents and minimize exposure to room temperature conditions.

Under aseptic conditions, combine the following reagents in the designated quantities:

a. DMEM 450 mL
b. 10% Fetal Bovine Serum (FBS)
c. 2 mM Glutamine 5 mL
d. 100 µL Penicillin/Streptomycin

2. Be sure to thoroughly mix both the Glutamine and Pen/Strep solutions prior to their addition. This can be accomplished through the use of the OHAUS Analog Mini Vortex Mixer (30392115).

3. Gently mix the solution through either inversion or serological pipette. Keep the mixture at 4 °C until use. Warm the media to 37 °C prior to treating cells. This can be accomplished through the use of the OHAUS Digital Dry Block Heater (30392080) and Sand Block Attachment (30400174). A water bath can also be used however it will increase the risk of contamination.

Initial Plating of Mammalian Cell Cultures

1. Obtain mammalian cells from either a biological or commercially available source.

2. Determine if the cells rely on suspension or are adherent.

3. If frozen, quickly thaw the cells and minimize their exposure to room temperature conditions.

4. Prepare the sample vessel by adding the desired amount of prepared media. For both adherent and suspension cells, a 50 mL culture flask will often suffice.

5. Using the recommended cell density, pipette the entirety of the initial culture into the appropriate vessel. Gently swirl the culture to ensure an even distribution of cells.

6. Immediately place the prepared culture into an incubator at 37 °C with 5% humidity.

7. Check the cells daily to ensure that they are healthy. Some cell death should be observed upon the initial plating.

Splitting of Cells (without the use of Trypsin or EDTA)

1. Allow the initial culture to become 80% confluent.

2. Determine the ratio at which the cells should be split. This is often dependent on when the cells will be used. Reference the guide below for a recommendation.

a. 1:2 split should be 70-80% confluent and ready for an experiment in 1 to 2 days.
b. 1:5 split should be 70-80% confluent and ready for an experiment in 2 to 4 days.
c. 1:10 split should be 70-80% confluent and ready for sub-culturing or plating in 4 to 6 days

3. Remove the cells from the incubator and place into an aseptic environment.

4. If cells are adherent, gently remove the initial media from the flask through either a pipette or gentle pour.

5. Wash the cells with warm phosphate buffered saline (PBS) by adding, then quickly removing, a thin layer directly onto the cells.
6. After completely removing the PBS, add fresh, warm media onto the cell culture at the initial volume.
7. Prepare the new flask that will be used for the split. Leave out media volume to allow for the addition of new cells. For example, if a 1–10 split is desired, add 9 mL of media, allowing for 1mL of cell containing media to be added later. Label the flask with the date and passage number, if it is the first time splitting the cells, the passage number would be 2.
8. Using a cell scraper, gently scrape the monolayer to release the adherent cells into the media. Ensure that all cells are detached prior to moving on the next step.
9. Once all cells are suspended, remove the desired volume needed for the split and immediately place into the freshly prepared flask. Gently swirl and place into an incubator at 37 °C with 5% humidity.
10. For suspension cells, skip steps 4, 5, 7 and 8 and remove the desired amount of cells directly from the initial culture and place into a 15 mL tube.
11. Centrifuge the tube at 1500 RPM for 3 minutes at room temperature using the OHAUS FC5714 Multi Pro Centrifuge (30314811) with a Swing Out Rotor (30314822 + 30314850)
12. Ensure that the cells have formed a pellet at the bottom of the tube then gently remove the supernatant.
13. Using a pipette, gently re-suspend the cells in fresh, warm media, and place directly into the freshly prepared flask. Gently swirl and place into an incubator at 37 °C with 5% humidity.
14. All left over cells can be either discarded or further passaged to create additional sub-cultures.

OHAUS Products Used Within This Procedure

Analog Mini Vortex Mixer

Digital Dry Block Heater

Sand Block Attachment

FC5714 Multi Pro Centrifuge

Swing Out Rotor