INTRODUCTION

Falcon Cell Culture Multi-Flasks are available in 3- and 5-layer formats and provide 525 cm$^2$ or 875 cm$^2$ cell growth surface areas, respectively, which is equivalent to 3 or 5 times the surface area of a T-175 flask. Falcon Multi-Flasks offer the same benefits as the current format but in a more efficient and higher capacity design enabling you to grow more cells faster, easier, and in a more convenient manner. The footprint is the same as the T-175, so adaptation to your current equipment should be simple. This device allows you the benefit of preparing your cell suspension within the flask and removing your media or reagents using a pipet. This reduces the risk of contamination and speeds up the procedure.

ADDING MEDIA AND PREPARING CELL SUSPENSION WITHIN THE FALCON MULTI-FLASK

1. Add required amount of medium into Falcon Multi-Flask by pipet or by pouring using typical culture volumes of 25-50 mL per layer.

2. To avoid foaming of medium, allow liquid stream to flow along the slope of the Logo side of the Falcon Multi-Flask.

   Helpful hint: A 10 mL pipet allows media to be dispensed at the bottom of the vessel. 25-100 mL pipets allow media to be dispensed just past the Logo.

3. Dispense cell suspension from a concentrated stock into the growth medium using a Falcon 10 mL pipet (Cat. No. 357551 or 356551). Be sure to dip the pipet tip into the medium.

   Note: The seeding density will vary depending on the cell type, medium, and culture duration needs. Begin with the same seeding density on a cells per centimeter square area to that used in standard flask for the cell type used.

4. Mix position: Hold the Falcon Multi-Flask upright with the Logo facing you and tilt counter-clockwise to a 45° angle.

5a. Holding at the same angle, gently rotate the Falcon Multi-Flask forward (neck pointing away from you).

5b. Then, gently rotate it backward (neck pointing towards you).

   Note: With each tilt, hold until liquid in the top layer drains fully.

6. Repeat Step 5 to ensure proper mixing. Bring back to mix position, as shown in Step 4. Then, proceed to Step 7 for equilibration.

7. After mixing or adding cell suspension, place the Falcon Multi-Flask vertically on a flat work surface to equalize liquid volume among all the layers.

ALTERNATE PROTOCOL

Adding cell suspension prepared external to the Falcon Multi-Flask

1. Create cell suspension externally from the Falcon Multi-Flask.

2. Add required amount of cell suspension into the Falcon Multi-Flask by pipet or by pouring.

3. To avoid foaming of medium, allow liquid stream to flow along the slope of the Logo side of the Falcon Multi-Flask.
PARTITION AND DISTRIBUTE LIQUID ONTO EACH Layer

8. Hold the Falcon® Multi-Flask upright with the Logo facing you and tilt clockwise to a 45° angle on a flat work surface to partition the liquid into each of the layers. This position is recommended for transport.

9. While holding the Falcon Multi-Flask at a 45° angle, gently lay it flat onto the work surface with Logo facing up.

10. After placing the Falcon Multi-Flask flat on a work surface, gently rock back and forth and side-to-side to distribute cells evenly onto culture surfaces – taking care not to spill liquid from each layer.

MEDIA REMOVAL

If exchange of media is required, follow Steps 7-10. You may choose to either aspirate or pour the media from the Falcon Multi-Flask.

ASPIRATING METHOD

11. To aspirate or remove media tilt the Falcon Multi-Flask, with the Logo facing you, counter-clockwise to a 45° angle while inverting the Multi-Flask toward you.

12. Then, tilt the Falcon Multi-Flask to the right, continuing to aspirate all residual media.

Helpful hint: Aspirate media using a Falcon 2 mL aspirating pipet (Cat. No. 357558).

POURING METHOD

13. With Logo facing down, pour spent media from the Falcon Multi-Flask.

Helpful hint: Pouring is easier when the Logo is facing you and the multi-flask is tilted at a counter-clockwise 45° angle.

CELL HARVESTING

14. Add dissociating reagent (> 5 mL per layer) based on preferred protocol and bring to mix position (Step 4). Then, follow Steps 7-10.

15. Neutralize with inactivating solution and mix following Steps 4-10. Gently swirl to dislodge cells completely.

16. Pipetting Method: Follow “Media Removal” protocol but collect cell suspension using a Falcon 10 mL serological pipet (Cat. No. 357551).

17. Follow Step 13 “Pouring Method”. Pour detached cell suspension into a Falcon conical tube (Cat. No. 352070).

18. Rinse with additional media as needed.

Helpful hint: Pouring is easier when the Logo is facing you and the multi-flask is tilted at a counter-clockwise 45° angle.