

Why does media spill over to the other layers when moving the Falcon Multi-Flask in and out of the incubator?

Transport the flask according to the user guide (see step 8) using the partition position and keeping fluid away from the mix port. Do not carry Falcon Multi-Flasks in a horizontal position as this may cause spillover of media between layers.

Why does media distribution become uneven when I place the Falcon Multi-Flask in the incubator?

Fluid may migrate from one layer to the other. To prevent this from occurring, one should:

- Ensure the incubator shelf is level.
- Position flasks across shelf without blocking the vented neck region.
- Ensure weight is distributed evenly throughout the shelf and the shelf can withstand the weight of flasks with media.
- Use the opposite corner of the mix port to pivot the flask and lay it horizontally. Ensure media does not flow back into the mix port or over the shelves into the neck region.

I followed the user guide and set the Falcon Multi-Flask into the incubator. A few hours later, I see uneven media distribution by layer. What do I do?

- Repeat re-equilibration and partitioning steps (user guide steps 7 and 8).
- Ensure the work surface is flat while equilibrating and partitioning fluid in the Falcon Multi-Flask.
- Inspect the flask at least 2 hours after seeding. Re-equilibrate and partition again, if necessary.
- Minimize vibration on, or near, the surface where Falcon Multi-Flasks are housed.

What volume of media should I use?

Begin with the same media volume and cell seeding density on a per unit surface area basis as your existing device. The surface area of the flasks are 525 cm² for 3-layer and 875 cm² for 5-layer flasks. The maximum amount of media used should not exceed 50 mL per layer.

What volume of dissociation reagent should I use?

We recommend ≥ 5 mL per layer of Falcon Multi-Flask.

Can I use the same volume of reagents as T-175 flasks?

No, the amount of media per unit area should be the same—not the actual total volume. If you currently use a T-175 flask, simply multiply the volume by 3 or 5 times depending on the Falcon Multi-Flask format used.

Why do I observe excessive frothing or bubbling of media and can this affect my cell culture?

Over mixing can result in excessive frothing or bubbling of media, which can lead to fluid migration from one layer to another. To avoid this, follow user guide step 5. While the flask is in the mixing position, allow the media to drain only from the layer on top; immediately tilt the flask in the opposite direction; and repeat. Ensure the flask is brought back to the mix position (user guide step 4) before equilibration of media. Improper mixing or pouring of media into the flask can also create foaming or bubbles. The correct way to add media is to follow step 1 in the user guide and pour or pipet gently along the slope of the flask lid.

How can the flasks be stacked in an incubator?

Ensure the flasks lock into position using the stacking ribs. Position the stacks of flasks evenly in the incubator to prevent the shelves from bowing. Allow adequate room for air circulation through the vented cap of the flasks. When stacking the Falcon® Multi-Flask on incubator shelves that are above shoulder height, ensure the media distribution by layer remains equal. The use of a step-stool can help facilitate flask placement.

What is the best way to add cells to the vessel?

Add the desired volume of growth media to the flask and keep it in an upright position. Add cell suspension stock (at least 10% final volume) directly into the growth medium using a 10 mL pipet.

Is pre-wetting of the flask or pre-warming of the media in the flask required?

Neither is required, nor are they recommended unless you are applying a coating to the flask growth surface.

The 5-layer Falcon Multi-Flask will not fit under my microscope. What do you recommend?

With some inverted microscopes, the condenser can be removed or moved to accommodate the 5-layer flasks. If this is not possible, a control flask (T-175) can be run side-by-side to provide an indication of cell morphology and confluence.

Why is cell growth patchy when I view the Falcon Multi-Flask under the microscope?

Uneven cell/media distribution most likely occurs from inadequate mixing. Refer to steps 4 and 5 of the user guide for the proper procedure. Don't use a clumpy cell suspension stock. Ensure cells are adequately triturated to generate a single cell suspension.

Why am I getting media in the neck of the flask?

Media should not enter the neck of the flask if the Falcon Multi-Flask is used properly. Check to ensure the flask is not upside down. The logo should face up when the flask is laid horizontally for incubation. Over-rotation when mixing cells in the vessel can result in media getting into the neck area of the flask. To avoid this, please follow steps 4 to 6 in the user guide.

Why are my cells not attaching?

If your cells normally attach to a single layer flask, then they will attach to the surface of the Falcon Multi-Flask. Ensure the flask is laid in the correct position with the logo facing up. In this position, cells will adhere to the tissue culture surface on the base of each layer.

Can I pour my cell suspension into the flask?

Yes, however we recommend that to avoid foaming of media, use a 50 or 100 mL pipet (Cat. Nos. 357550 and 357600). Insert the pipet past the logo and stream liquid along the top slope of the flask into the first layer.

Can I pour my cells and reagents out of the flask?

Yes, you can pour or use a pipet to recover the cells during harvesting. If cells clump, mechanically dissociate the cells by titrating the suspension.

How can I maximize the recovery of cells and reagents?

- To minimize residual volumes, pour as per step 13 in the user guide. Allow liquid to pool on the mix port side prior to pouring with the logo down.
- For maximal recovery of cells and reagents, follow steps 11 and 12 in the user guide. First, aspirate by positioning the pipet tip towards the mix port region. Then, pool liquid into the top layer and tilt the flask to partition liquid away from mix port.
- For high density cell cultures, additional rinses are recommended. Visually inspect the base layer to determine how many rinses are required.

Why am I not getting the expected cell yield?

- You may not be recovering all the cells. Additional rinses may be required, as noted above.
- You may have uneven distribution of cells and media. See the user guide for proper mixing, equalization and partitioning procedures.
- You should avoid lengthy delays between cell seeding and incubation so that cells do not settle to the bottom of the vessel.

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