

# 5-Layer Cell Culture Flasks

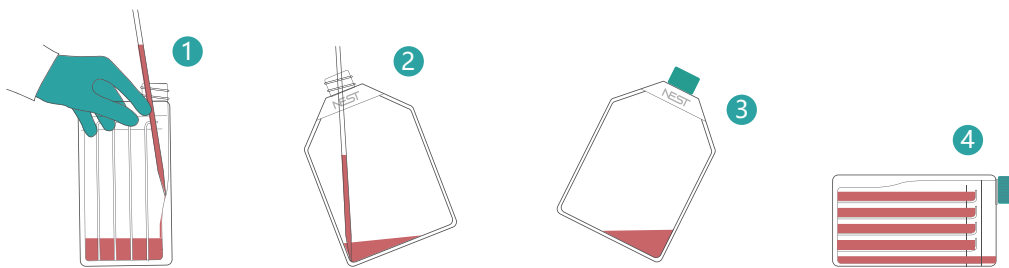


## Features

- Made of high clarity, 100% virgin polystyrene.
- Sterilized by E-beam, SAL=10<sup>-6</sup>.
- Non-Pyrogenic, DNase/Rnase free.
- Vacuum plasma tissue culture treated (TC treated).
- Growth area: 870 cm<sup>2</sup>.
- Individually Packaged in sterile bag.

Cat.No.	Cap Style	Recommended Medium Volume(mL)	TC	/Pack	/Case
731001	Plug Seal Cap	100-150	Yes	1	8
731002	Vent Cap	100-150	Yes	1	8

## Guidelines For Use:



1. Mix cell suspension with medium: Prepare cell suspension of required concentration in a container. Recommended volume is about 30- 50mL per layer.
2. Add the mixed liquid into the Multi-layer Flask slowly with serological pipettes. To avoid foam and bubbles, allow liquid stream to flow along the slope of the Multi-layer Flask. (Save a little liquid in pipette each time.)
3. Tips: A 10 mL pipette allows media to be dispensed at the bottom of the vessel. A 25 mL pipette allows media to be dispensed just past the NEST Logo.
4. Hold the Multi-layer 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 layer.
5. While holding the Multi-layer Flask at a 45° angle, gently lay it flat onto the work surface with logo facing up.
6. After placing the Multi-layer Flask flat on a work surface, gently rock back and forth and side-to-side to distribute cells evenly onto culture surfaces.
7. Tips: Be careful to avoid foaming of medium, and not to spill liquid from each layer.
8. Repeat Step 3 to put the flask quickly and slightly into the incubator. Then, lay it flat as shown in Step 4. You may choose to either aspirate or pour the media from Multi-layer Flask.
9. Aspirating method: To aspirate or remove media, tilt Multi-layer Flask, with the NEST Logo facing you, counter-clock wise to a 45° angle while inverting the Multi-Flask toward you. Then, tilt Multi-layer Flask to the right, continuing to aspirate all residual media.
10. Pouring method: With Logo facing you, pour spent media from Multi-layer Flask
11. Tips: Aspirate media using a NEST 2 mL or 10mL aspirating pipette.
12. Wash with buffer for one time and add dissociating reagent (≥5mL per layer). Then, follow Steps 3-4 to distribute to dissociating reagent to each layer.
13. Neutralize with inactivating solution and mix following Steps 3-4. Gently swirl to dislodge cells completely.
14. Follow Step 7 "Aspirating Method" protocol and collect cell suspension using a NEST 10mL serological pipette.
15. Follow Step 8 "Pouring Method". Pour the cell suspension into a NEST conical tube.
16. Rinse with additional wash buffer if needed.
17. Search "NEST Multi-layer Flask" video on NEST website or Youtube.