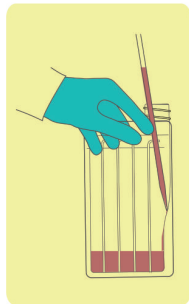


NEST Multi-Layer Flasks provide 870 cm<sup>2</sup> cell growth surface area. The tissue culture treated NEST Multi-layer Flasks enable you to grow more cells faster and easier, thereby making your cell culture workflow more productive, easier, and in a more convenient manner.

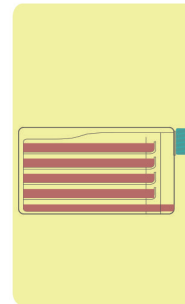
## ADDING MEDIA AND PREPARING CELL SUSPENSION:



1. Mix cell suspension with medium: Prepare cell suspension of required concentration in a container and mix. Recommended volume is about 30- 50 mL per layer.
2. Add the mixed liquid into the Multi-layer Flask slowly with serological pipettes. To avoid foams and bubbles, allow liquid stream to flow along the slope of the Multi-layer Flask. ( Save a little liquid in pipette for each dosing . )  
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.



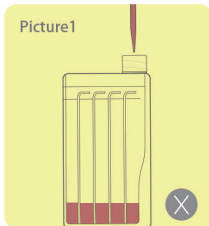
3. Hold the Multi-layer Flask up-right with the Logo facing you and tilt clockwise to a 45° angle on a flat work surface to partition the liquid into each layer.
4. While holding the Multi-layer Flask at a 45° angle, gently lay it flat onto the work surface with logo facing up.



5. 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.  
Tips: Take care to avoid foaming of medium, and not to spill liquid from each layer.
6. Repeat Step 3 to put the flask quickly and slightly into the incubator . Then, lay it flat as shown in Step 4 .

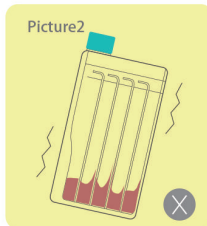
### Tips:

Picture1



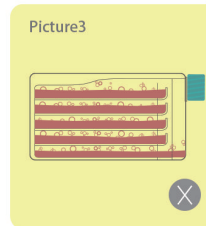
1. When adding the liquid into the Multi-Layer Flask, make sure the pipette tip into the Flask and close to the Flask inner wall. **Picture 1 shows the improper operation.**

Picture2



2. After adding the liquid, hold the Multi-Layer Flask and gently rock back and forth, **avoid violent shaking.**

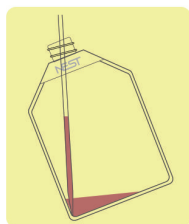
Picture3



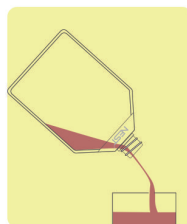
3. Take care to avoid foaming. Foaming will cause the medium flow to the next layer. **Do not repeat the foaming showed in Picture 3.**

## MEDIA REMOVAL:

You may choose to either aspirate or pour the media from Multi-layer Flask.



7. 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.



8. Pouring method :  
With Logo facing you, pour spent media from Multi-layer Flask  
Tips: Aspirate media using a NEST 2mL or 10mL aspirating pipette.

## PRODUCT SPECIFICATIONS:

WORKING VOLUME RANGE	≥5mL per layer for dissociating ≥30mL per layer for cell expansion
MOLDED-IN GRADUATIONS	0 to 50mL per layer in 10mL increments
GRADUATION ACCURACY	10%
CAP VENT MEMBRANE	0.22um hydrophobic membrane
CELL GROWTH SURFACE	tissue culture-treated, optically clear

## CELL HARVESTING:

9. Wash with buffer for one time and add dissociating reagent (≥5 mL per layer). Then, follow Steps 3-4 to distribute to dissociating reagent to each layer.
10. Neutralize with inactivating solution and mix following Steps 3-4. Gently swirl to dislodge cells completely.
11. Follow Step 7 "Aspirating Method" protocol and collect cell suspension using a NEST 10mL serological pipet .
12. Follow Step 8 "Pouring Method". Pour de-tached cell suspension into a NEST conical tube.
13. Rinse with additional wash buffer as needed.

Cat. No.	Description	/pack	/case
731001	5-layer cell culture flask, straight neck, plug seal cap, TC treated	1	8
731002	5-layer cell culture flask, straight neck, vent seal cap, TC treated	1	8

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