

# Cell Culture Flask

## Application

- Tissue culture
  - Stem cell research
  - Cell biology research
  - Cell therapy direction research
  - Small-scale cell culture, cell screening
  - Biochemistry, medicine, microbiology
  - Cytology research in animal and plant and basic medical school
  - Genetic engineering, antibody engineering drugs, vaccines, etc.
- Microplate chromogenic reaction plate use



T150 U-Shaped Canted Neck Cell Culture Flask



Cell Culture Flask Closed System Solution



Cell Culture Flask

NEST's Cell Culture Flasks whose cell growth areas ranging from 25cm<sup>2</sup> to 225cm<sup>2</sup> are available. These flasks are available as issue culture treated or non-treated as well as with a vent cap or plug seal cap to meet your requirements.

## ● Features

- Made of high clarity, 100% virgin polystyrene.
- Sterilized by E-beam, SAL=10<sup>-6</sup>.
- Non-Pyrogenic, DNase/Rnase free.
- Frosted writing and clear graduations.
- Notched bottom for slip free slip stacking.
- Clear lot number for batch traceability.
- Packaged in sterile, zip-sealable bags.

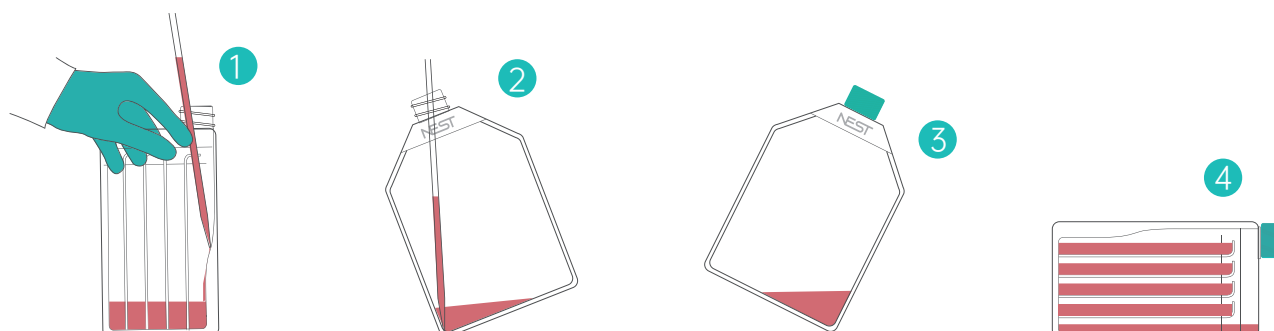
## ● 3 / 5-layer Cell Culture Flask

- Made of high clarity, 100% virgin polystyrene.
- Sterilized by E-beam, SAL=10<sup>-6</sup>.
- Non-Pyrogenic, DNase/Rnase free.
- 3-layer Cell Culture Flask Growth area: 520 cm<sup>2</sup>.
- 5-layer Cell Culture Flask Growth area: 870 cm<sup>2</sup>.
- Individually packaged in sterile bag.





## Protocols for seeding cells into a 5-layer Cell Culture Flask



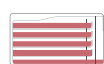
1. Prepare cell suspension of the required concentration, then mix it with medium evenly in a container. A volume of 30-50 mL per layer is recommended.
2. Slowly add the mixed solution into the 5-layer Cell Culture Flask with a serological pipette. To avoid foam or bubbles, it is recommended to set the pipette firmly against the wall, enable the stream to flow along the slope, and save a little liquid in the pipette each time.  
*Notes: While a 10 ml pipette can disperse the medium at the bottom, a 25 ml pipette will only reach up to the NEST mark to disperse the medium.*
3. Position the Multi-layer Flask upright with the NEST mark facing you, tilt it 45° clockwise and let stand in this position for a while to level the liquid in each layer.
4. Gently lay it flat onto the workbench with NEST mark facing upwards.
5. Gently shake it from side to side to distribute cells evenly onto culture surfaces.  
*Notes: be careful to shake gently to avoid foam or bubbles and spilling liquid from each layer.*
6. Transfer the flask to the incubator for incubation

### ● Culture medium removal

1. Aspiration  
Tilt the flask 45° clockwise with the NEST mark facing you. count-er-clock wise to a 45° angle while inverting the Multi-Flask toward you. Then reach the serological pipette into the bottom for fully aspiration.
2. Pouring  
Tilt the flask 45° counterclockwise with the NEST mark facing you, pour the spent media from the flask.  
Tips: A NEST 10mL serological pipette is suggested for fully aspiration

### ● Cell Culture Flask

Cell Growth Area (cm <sup>2</sup> )	Maximum capacity (mL)	Recommended Medium Volume (mL)	Size(mm)			Packing		TC Treated		Non-Treated	
			Bottom height	Width	Length	/Pack	/Case	Plug seal caps	Vent caps	Plug seal caps	Vent caps
25	30	5-7.5	25.9	53.8	97.13	10	20	707001	707003	707011	707013
75	225	15-22.5	35.7	89.56	160.01	5	20	708001	708003	708011	708013
150	375	30-45	40.3	110.75	203	5	8	720001	720003	720011	720013
175	400	35-52.5	39.1	120.51	217.9	5	8	709001	709003	709011	709013
225	700	45-67.5	46.05	137	238.5	5	5	721001	721003	721011	721013
520 ( 3-layer)	50	60-100	60.1	120.5	203	1	12	731301	731302	/	/
870 ( 5-layer)	50	100-150	84.3	120.5	203.6	1	8	731001	731002	/	/



### ■ Precautions:

It is important to handle multi-layer Cell Culture Flasks with caution to avoid the formation of bubbles. The presence of bubbles can lead to the creation of siphon bridge at the baffle, resulting in the upper layer of culture medium flowing down to the bottom.

### ● Cell harvesting

1. Rinse off the residual serum with buffer, add digestion solution ( $\geq 5$  mL per layer) and mix evenly. Then, follow Steps 3-4 to distribute to dissociating reagent to each layer.
2. Let stand for 2 min, then neutralize and mix with inactivating solution following steps 3-4. Gently swirl to dislodge cells completely.
3. Transfer the solution in a centrifuge tube or other containers by aspiration or pouring.
4. Rinse the flask with buffer for three times, then transfer the buffer into the centrifuge tube for passage and counting.  
Tips: Search "NEST Multi-layer Flask" video on YouTube(@nestwuxi4075).

## Cell Culture Flask Closed System Solution



### 1 Filter

The Pre-installed filter on the cap facilitates gas exchange during liquid transfer, and allows for subsequent cell culture.



### 2 Clamp

Clamp should keep open for gas exchange during liquid transfer. After the transfer is complete, it can be closed for the growth of anaerobic cells and viruses.



### 3 Thermoplastic Elastomer Tube (TPE Tube)

TPE tube can be connected to the tubing system via a tube sealing machine under non-sterile conditions.



### 4 PTFE Tube


PTFE tubing is placed inside the bottle below the filter to facilitate gas exchange during liquid transfer.



The range of T-flask Closed System allow for aseptic transfer of liquids, during which culture media or cells can be transferred into or out of bioreactors or BioFactorie without the need to open the cap. After the transfer, the culture media and cells can be directly cultured inside the flask, eliminating the exogenous contamination risk for drug development and other production processes. In addition, it also reduces the unnecessary time wasted for tubing designing, assembly, and sterilization, thereby improving production efficiency.

### ● Removal of culture media

- Allows for cell culture and liquid transfer without the need to open the cap and hence minimizes the risk of contamination during liquid transfer.
- Intended for both aerobic cells (by opening the clamp) and anaerobic cells (by closing the clamp).
- Liquid transfer can be achieved by gravity without the need for peristaltic pumps.
- The one-piece injection moulding for bottle and cap reduces the risk of leakage and media residue.
- The TPE tube can be sealed in non-sterile environments.
- The high-quality materials makes smooth inner wall of the tube and ensures excellent transfer performance.
- Sterility assurance level (SAL)=  $10^{-6}$ .
- Free from endotoxins and animal-derived components.

Growth Area (cm <sup>2</sup> )	Tubing Measurements			Membrane Area of 0.22µm Filter	/Case	Cat.NO.
	Diameter	Length	Joint Connection			
25	1/4" ID, 3/8" OD	40cm	aseptic welding /heat sealing	4.5cm <sup>2</sup>	4	C92032-BZB040A
75						C92131-BZB040A
225						C92231-BZB040A