

NEST® 多层细胞培养瓶 技术手册

需要培养大量的细胞，忙得焦头烂额，又担心污染？别急，现在有了新工具来帮忙。NEST® 多层细胞培养瓶采用医用级聚苯乙烯材料制作，提供更大培养面积。五层细胞培养瓶瓶总面积为 870cm²，三层细胞培养瓶瓶总面积为 520cm²。它能够帮助您更快、更方便地培养细胞，从而使细胞培养工作更为有效。

细胞悬液接种到多层细胞培养瓶：

培养液的移除：

你可以选择抽吸或者倾倒培养的方法去除培养基。

1. 细胞悬液混匀：按照实验要求的浓度准备细胞，在培养基瓶、培养瓶或其他容器中混匀。建议的工作容积为每层 30-50mL。

2. 用移液管紧贴瓶壁缓慢地将液体加到多层细胞培养瓶中，避免产生泡沫或气泡（每次加液时移液管内液体可留少许）。

注意事项：10mL 移液管可以伸到培养瓶底部使培养基分散开，而 25 mL 移液管则只能伸到 NEST 标志的地方把液体分散开。

3. 把培养瓶有 NEST 标志那一侧朝向自己，顺时针倾斜 45 度左右放置一段时间，使瓶中各个隔层的液体达到平衡。

4. 轻缓地将培养瓶水平放置于工作台上，有耐思标志那一面朝上。

5. 前后左右轻轻晃动多层瓶，液体会均匀地分布在每层生长面上。
注意事项：摇晃的动作要轻柔，一方面是防止起泡沫，另一方面是要防止培养基渗漏到下层中。

6. 水平轻缓转移置于 CO₂ 培养箱。

细胞的收集：

9. 用缓冲液清洗一遍以去除残留的血清，后加入消化液（≥5mL / 层），混匀。

10. 消化大约 2 min（视细胞情况而定），用消化终止液中和消化液。

11. 采用移液管抽吸法或倾倒法将细胞悬液置于离心管等容器中。

12. 用一定量的缓冲液冲洗多层瓶三次，将冲洗液同时置于离心管中，洗计数或者传代接种。

产品信息

料号	描述	/袋	/箱
731001	5 层细胞培养瓶, 密封盖	1	8
731002	5 层细胞培养瓶, 透气盖	1	8
731301	3 层细胞培养瓶, 密封盖	1	12
731302	3 层细胞培养瓶, 透气盖	1	12

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NEST Multi-Layer Cell Culture Flask Culturing Technique

NEST multi-layer flasks provide 174 cm² cell growth surface area per layer. 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.

7. Aspirating Method:
To aspirate or remove media, with the NEST logo facing you, tilt the multi-layer flask counter-clockwise to a 45° angle while inverting the multi-layer flask toward you. Then, tilt multi-layer flask to the right, continuing to aspirate all residual media.
Tips: Aspirating media completely requires the usage of a NEST 2mL or 10mL serological pipette.

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 to prevent bubbles.)
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 at the spot just past the NEST logo.

3. 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.

4. While holding the multi-layer flask at a 45° angle, gently lay it flat onto the work surface with the 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. Gently horizontally transfer and place the flask to a CO₂ incubator.

MEDIA REMOVAL

You may choose to either aspirate or pour the media from the multi-layer flask.

8. Pouring Method:
With the logo facing you, pour the media from the multi-layer flask.

CELL HARVESTING

9. Wash with buffer once and add dissociating reagent (≥5 mL per layer). Then, follow Steps 3-4 to distribute the dissociating reagent to each layer.

10. Neutralize with inactivating solution and mix it following Steps 3-4. Gently swirl to dislodge cells completely.

11. a) Follow Step 7 "Aspirating Method" protocol and collect cell suspension using a NEST 10mL serological pipette.
b) Follow Step 8 "Pouring Method". Pour the detached cell suspension into a NEST conical tube.

12. 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 cap, TC treated	1	8
731301	3-layer cell culture flask, straight neck, plug seal cap, TC treated	1	12
731302	3-layer cell culture flask, straight neck, vent cap, TC treated	1	12

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 0.22μm hydrophobic membrane
CELL GROWTH SURFACE tissue culture-treated, optically clear

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