EpiCult™ Plus Medium

For culture of human, mouse, and rat epithelial stem cells

Catalog #06070 500 mL

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Product Description

EpiCult™ Plus Medium is a serum-free and BPE-free culture medium for the expansion of epithelial stem cells from mammary, prostate, and other stratified and pseudostratified epithelial tissues from human, mouse, and rat. Epithelial cells can be expanded in EpiCult™ Plus Medium for multiple passages and for > 30 population doublings; for some human mammary cells, addition of fetal bovine serum may be required for extended passaging. EpiCult™ Plus Medium is not recommended for human keratinocytes or primary human airway cells; for culturing primary human airway cells, we recommend PneumaCult™-Ex Plus Medium (Catalog #05040).

NOTE: Hydrocortisone Stock Solution (Catalog #07925) is required for preparation of complete EpiCult™ Plus Medium.

Product Information

The following components are sold as a complete kit (Catalog #06070) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
EpiCult™ Plus Basal Medium	06071	490 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
EpiCult™ Plus Supplement*	06072	10 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

*This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Hydrocortisone Stock Solution	07925
Collagen I-coated 6-well tissue culture plate	e.g. Corning 356400
D-PBS (Without Ca++ and Mg++)	37350
Trypsin-EDTA (0.25%)	07901
Fetal bovine serum (FBS; quality cell culture-tested)	---
15 mL conical tubes	38009
Trypan Blue	07050

Preparation of Complete EpiCult™ Plus Medium

Use sterile technique to prepare complete EpiCult™ Plus Medium (Basal Medium + Supplement + hydrocortisone). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw EpiCult™ Plus Supplement at room temperature (15 - 25°C). Mix gently by inverting the vial; do not vortex.

   NOTE: A precipitate may be observed after thawing; this will not affect performance.

   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the expiry date as indicated on the label. Once aliquots are thawed, use immediately. Do not re-freeze.
2. Add 10 mL of EpiCult™ Plus Supplement and 0.5 mL Hydrocortisone Stock Solution to 490 mL of EpiCult™ Plus Basal Medium. Mix gently by inverting the bottle.

   NOTE: Complete medium does not contain antibiotics; add antibiotics if desired.

   NOTE: If not used immediately, store complete EpiCult™ Plus Medium at 2 - 8°C for up to 4 weeks. Do not exceed the shelf life of the individual components.

Directions for Use

Please read the entire protocol before proceeding.

The following protocol is for expanding cultured primary epithelial cells in a single well of a collagen I-coated 6-well tissue culture plate. For other cultureware sizes, adjust cell numbers and volumes accordingly.

NOTE: Human mammary cells from some donors may be difficult to maintain in culture for extended periods (> 15 population doublings); to overcome this, add 2% FBS to complete EpiCult™ Plus Medium throughout the protocol below.

1. Day 0: Add 8 - 10 x 10^4 cells in 2.5 mL complete EpiCult™ Plus Medium to one well of a collagen I-coated 6-well plate. Incubate at 37°C for 3 days.

   NOTE: If using cryopreserved cells, wash cells once with complete EpiCult™ Plus Medium prior to plating.

2. Day 3: Perform a full medium change by removing medium from the well and adding 2.5 mL of complete EpiCult™ Plus Medium. Incubate at 37°C. Perform a full medium change every 3 days. When cells reach 50 - 70% confluency (approximately Day 4 - 10), they are ready for passaging.

   NOTE: On weekends, perform a full medium change on Friday afternoon and first thing on Monday morning.

3. Passage cells as follows:
   a. Warm complete EpiCult™ Plus Medium and Trypsin-EDTA (0.25%) to 37°C.

      NOTE: If using EpiCult™ Plus Medium + 2% FBS, additionally warm D-PBS to 37°C.

   b. Remove the plate from the incubator and remove the medium from the well.

      NOTE: If using EpiCult™ Plus Medium + 2% FBS, rinse the well with 3 mL of warm D-PBS.

   c. Add 1 mL of warm Trypsin-EDTA to each well.
   d. Incubate at 37°C for 4 - 6 minutes.
   e. Remove the plate from the incubator. Using a 1 mL pipettor set to 1 mL, pipette the Trypsin-EDTA up and down several times to detach cells.
   f. Add 3 mL of D-PBS + 4% FBS per well to inactivate the trypsin.

      NOTE: For a serum-free system, use 3 mL of a 1 mg/mL stock solution of Soybean Trypsin Inhibitor, ACF (Catalog #07457) instead of D-PBS + 4% FBS.

   g. Transfer the cells to a 15 mL conical tube. Centrifuge at 300 x *g* for 5 minutes.
   h. Discard the supernatant and resuspend the cell pellet in 0.5 mL complete EpiCult™ Plus Medium.
   i. Perform a viable cell count using Trypan Blue and a hemocytometer.
   j. Add 2 - 5 x 10^4 cells in 2.5 mL complete EpiCult™ Plus Medium to a new collagen I-coated 6-well plate. Gently move the plate in several quick, short, back-and-forth and side-to-side motions to evenly distribute the cells.

4. Incubate at 37°C. When cells reach 50 - 70% confluency (approximately 3 - 5 days), they are ready for passaging.

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