

HuMEC Ready Medium (1X)

Description

HuMEC Ready Medium (1X) has been developed for the growth and expansion of human mammary epithelial cells (HuMEC). HuMEC Ready Medium is a complete serum-free medium consisting of HuMEC Basal Serum Free Medium (SFM) with the addition of HuMEC Supplement and Bovine Pituitary Extract (BPE).

Product	Catalog No.	Amount	Storage	Shelf Life*
HuMEC Ready Medium	12752-010	1 kit		
Contains:				
HuMEC Basal Serum Free Medium	12753-018	1 × 500 mL	2°C to 8°C; Protect from light	18 months
HuMEC Supplement	12754-016**	1 × 5 mL	-20°C to -5°C; Protect from light	18 months
Bovine Pituitary Extract (BPE)	13028-014	1 × 25 mg	-20°C to -5°C	18 months
HuMEC Supplement Kit	12755-013	1 kit		
Contains:				
HuMEC Supplement	12754-016**	1 × 5 mL	-20°C to -5°C; Protect from light	18 months
Bovine Pituitary Extract (BPE)	13028-014	1 × 25 mg	-20°C to -5°C	18 months
HuMEC Basal Serum Free Medium	12753-018	1 × 500 mL	2°C to 8°C; Protect from light	18 months

* Shelf Life duration is determined from Date of Manufacture.

** HuMEC Supplement is not sold separately.

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Important Information

Avoid repeated freeze/thaw cycles of HuMEC Supplement. Use immediately once thawed.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare Medium

HuMEC Basal Serum Free Medium requires supplementation with HuMEC Supplement and BPE.

1. Aseptically add 5 mL of HuMEC Supplement to 500 mL of HuMEC Basal Medium before use.
2. Aseptically add 25 mg of Bovine Pituitary Extract to the medium before use.
3. Add antibiotics, if required. We recommend Penicillin-Streptomycin or Gentamicin-Amphotericin at 10–50 µg/mL.

Once supplemented, the complete HuMEC Ready Medium is stable for up to 15 days when stored in the dark at 2 to 8°C.

Culture Conditions

Media: Complete HuMEC ready Medium.

Cells: Human mammary epithelial cells (HuMEC).

Culture Type: Adherent

Culture Vessels: T-flasks.

Temperature Range: 36°C to 38°C.

Incubator Atmosphere: Humidified atmosphere of 4–6% CO₂ in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Recovery

1. Rapidly thaw (<1 minute) frozen vial of cells in a 37°C water bath.
2. Gently pipet cell suspension up and down several times using a wide-bore pipette to ensure a homogeneous suspension. Determine total viable density and cell viability using a Countess[®] Automated Cell Counter (alternate automated or manual methods may be used).
3. Dilute cell suspension to 1.25 × 10⁴ viable cells/mL with complete HuMEC Ready Medium. Add 5 mL cell suspension per 25 cm² to each tissue culture flask. Swirl cell suspension in each flask to evenly distribute cells.
4. Incubate at 37°C in a humidified atmosphere of 5% CO₂ in air. Loosen flask caps (vented caps may be used) to allow for gas exchange.
Note: For best results do not disturb cells for at least 18 hours after plating.
5. Exchange spent media with fresh complete HuMEC Ready Medium 24 hours after plating and every 48 hours thereafter. Subculture cells 3–5 days post-thaw.

Subculture Cells

Subculture human mammary epithelial cells directly into complete HuMEC Ready Medium. Ensure that the cell confluency is between 40–80%, cell viability is at least 90%, and growth rate is in mid-logarithmic phase prior to subculturing. For optimal performance and cell growth, re-feed cultures every 3–4 days with fresh medium.

Note: Procedures are for cultures in a T-75 flask (75 cm²). Adjust volumes accordingly to culture vessel size.

1. Observe cell monolayer to ensure confluence (40–80%). Aspirate medium and floating cells from monolayer and discard.
2. Add 5–10 mL Dulbecco's Phosphate Buffered Saline (DPBS), without calcium and magnesium to culture flask. Gently wash cell monolayer.

- Remove DPBS and add 5–7 mL of prewarmed TrypLE™ Express cell dissociation reagent, without phenol red to the monolayer.
- After 2 minutes, remove the TrypLE™ Express reagent and incubate flask 37°C for approximately 10–15 minutes or until cells have fully detached.
- Observe cell monolayer using an inverted microscope to ensure complete detachment from the surface of the flask.
- Add 5–7 mL of prewarmed complete HuMEC Ready Medium to the flask to resuspend the cells.
- Remove cell suspension from flask and transfer into a conical tube.
- Determine total viable density using a Countess® Automated Cell Counter or alternative automated or manual method.
- Inoculate flask at $3\text{--}5 \times 10^3$ viable cells/cm².
- Incubate at 37°C in a humidified atmosphere of 5% CO₂ in air.
- Day 3 or 4 post-plating, carefully aspirate the spent medium from cell monolayer and add fresh medium without disturbing cell monolayer. Return flask(s) to the incubator.

Cryopreservation

- Prepare the desired quantity of cells in a tissue culture flask, harvesting in mid-log phase of growth with viability >90%. Reserve the conditioned medium to prepare cryopreservation medium.
- Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of $>1 \times 10^6$ cells/mL.
- Prepare the required volume of cryopreservation medium of 92.5% HuMEC Ready Medium (50:50 ratio of fresh-complete to conditioned media) +7.5% DMSO and store at 4°C until use.
Important: Prepare cryopreservation medium on the day of intended use.
- Harvest cells by centrifugation at $100 \times g$ for 5–10 minutes. Resuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.
- Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1 mL in a 2-mL cryovial).
- Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
- Transfer frozen cells to liquid nitrogen (vapor phase); storage at –200°C to –125°C is recommended.

Related Products

Product	Catalog No.
Dulbecco's Phosphate Buffered Saline, without calcium and magnesium	14190
TrypLE™ Express (1X), no Phenol Red	12604
0.05% Trypsin-EDTA (1X), Phenol Red	25300
Gentamicin/Amphotericin B (500X)	R-015-10
Penicillin-Streptomycin, Liquid	15140
Human Mammary Epithelial Cells (HMEC)	A10565
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

Temperature Limitation	Manufacturer	Batch code	Use By:
Caution, consult accompanying documents	Consult instructions for use	Sterilized using aseptic processing techniques	
Read Safety Data Sheet	Catalog number	Protect from light	

Limited Product Warranty

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For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email techsupport@lifetech.com

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