

## HuMEC Ready Medium

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

HuMEC Supplement:

**Caution: Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HB<sub>s</sub>Ag. Handle in accordance with established bio-safety practices.**

Description	Cat. No.	Size
<b>HuMEC Ready Medium</b> Contains: HuMEC Basal Serum Free Medium HuMEC Supplement* Bovine Pituitary Extract (BPE)	<b>12752-010</b>  12753-018 12754-016 13028-014	<b>1 Kit</b>  1x500 mL 1x5 mL 1x25 mg
<b>HuMEC Supplement Kit</b> Contains: HuMEC Supplement* Bovine Pituitary Extract (BPE)	<b>12755-013</b>  12754-016 13028-014	<b>1 Kit</b>  1x5 mL 1x25 mg
<b>HuMEC Basal Serum Free Medium</b>	<b>12753-018</b>	<b>500 mL</b>

*\*Note: HuMEC Supplement is not sold separately*

### Intended Use

For research use only. CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

### Features

- A serum-free 1X formulation designed for the culture of human mammary epithelial cells.
- Increase cell growth with consistent gene expression profiles.
- Improved consistency and reduced variability compared to conventional serum supplemented media.

### Preparation of HuMEC Ready Medium

HuMEC Basal Serum Free Medium requires supplementation with HuMEC Supplement and BPE. Complete medium is stable for 21 days when stored at 2 to 8° C in the dark.

- Aseptically add 5 mL of HuMEC Supplement to 500 mL of HuMEC Basal Medium before use.
- Aseptically add 25 mg of Bovine Pituitary Extract to the medium before use.
- If required, antibiotics can be used. It is recommended to use Penicillin/Streptomycin (Cat. No. 15140) or Gentamicin/Amphotericin (Cat. No. R-015-10).

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

### Storage

- HuMEC Basal Serum Free Medium: Store in the dark at 2 to 8°C
- HuMEC Supplement: Store in the dark at -5 to -20°C

### Shelf Life

HuMEC Basal Serum Free Medium: 12 months

HuMEC Supplement: 6 months

### Physical Conditions

Standard physical conditions for human mammary epithelial cells (HMEC) grown in complete HuMEC medium are 36 to 38°C in a humidified atmosphere of 4 to 6% CO<sub>2</sub> in air. Using standard aseptic conditions, cultures may be grown in tissue culture flasks (e.g. T-75 cm<sup>2</sup> flask). Ensure proper gas exchange is achieved in tissue culture flasks. Avoid overexposure of cultures to light.

### Recommended Protocols:

The following procedures are designed to be used with Invitrogen's Human Mammary Epithelial Cells (HMEC) (A10565). Further modification of the protocols may be required depending on human mammary epithelial cells used.

**Note: Unless noted, it is recommended not to pre-warm reagents prior to use.**

### Initiating Cultures from Cryopreserved Cells

It is recommended to seed HMEC recovered from cryopreservation at a density of 2.5 x 10<sup>3</sup> viable cells/cm<sup>2</sup>. For example, one vial containing ≥ 5 x 10<sup>5</sup> HMECs can seed approximately three 75 cm<sup>2</sup> or nine 25 cm<sup>2</sup> tissue culture flasks.

1. Prepare a bottle of supplemented HuMEC medium.
2. Remove a vial (5 x 10<sup>5</sup> HMEC /vial) of HMEC from liquid nitrogen storage, taking care to protect hands and eyes.
3. Lower bottom half of the vial into a 37°C water bath to thaw.
4. When the contents of the vial have just thawed, wipe the outside of the vial with disinfecting solution and move to a Class II, Type A laminar flow culture hood.

- Open the vial and gently pipette the suspension up and down with a 1 mL pipette to disperse the cells.
- Remove 20  $\mu$ l from the vial and dilute the cell suspension in 20  $\mu$ l of Trypan blue solution (Cat. No 15250).
- Use a hemacytometer to determine the number of viable cells/mL.
- Dilute the contents of the vial (1 mL) to a concentration of  $1.25 \times 10^4$  viable cells/mL using the supplemented medium.
- Add 5 mL of cell suspension to each 25 cm<sup>2</sup> culture flask or 15 mL of cell suspension to each 75 cm<sup>2</sup> culture flask.
- Following inoculation, swirl the medium in the flasks to evenly distribute the cells.
- Incubate the cultures in a 37°C, 5% CO<sub>2</sub>/95% air, humidified cell culture incubator. For best results, do not disturb the culture for at least 18 hours after the culture has been initiated.

### Maintenance of Stock Cultures

- Change the culture medium to freshly supplemented medium, 18 to 24 hours following plating of cryopreserved cells. For subsequent subcultures, change the medium 48 hours after establishing the subculture.
- Change the medium every other day thereafter, until the culture is approximately 50% confluent.
- Once the culture reaches 50% confluence, change the medium every day until the culture is approximately 80 to 90% confluent.

**Note:** To achieve the highest cell densities, the culture medium should be changed every day as the cultures approach confluence. To obtain rapidly proliferating subcultures, HMEC should be subcultured before they become more than 90% confluent. The number of subcultures (passages) that can be achieved will vary with the starting cell density and the methods employed by individual investigators.

HMEC cultures seeded at  $2.5 \times 10^3$  cells/cm<sup>2</sup> from cryopreserved cells should reach 80-90% confluence in 5 to 7 days. At this time, most of the cells should have epithelial or "cobblestone" morphology. Some irregularly sized and shaped cells may be observed.

### Subculture of HMEC

View the culture under the microscope to confirm that it is subconfluent (80 to 90%), and that there are mitotic cells present. This protocol is designed for the subculture of one T25 cm<sup>2</sup> culture flask. If different-sized culture vessels are to be used, reagent volumes should be adjusted accordingly.

- Prepare complete supplemented medium.
- Assemble the appropriate culture vessels, sterile pipettes, and sterile 15 mL conical tubes.
- Remove all of the culture medium from the flask.
- Add 3 mL of Trypsin/EDTA solution to the flask. Rock the flask to ensure that the entire surface is covered.
- Immediately remove all 3 mL of Trypsin/EDTA solution from the flask.
- Add 1 mL of fresh Trypsin/EDTA solution to the flask.
- Incubate the flask at 37°C for 4-6 minutes. View the culture under a microscope to confirm cells have become round.
- Rap the flask very gently to dislodge cells from the surface of the flask.
- Add 3 mL of Trypsin Neutralizer solution to the flask and transfer the detached cells to a sterile 15 mL conical tube.

- Add 3 mL of additional Trypsin Neutralizer solution to the flask and pipette the solution over the flask surfaces several times to remove any remaining cells. Add this suspension to the 15 mL conical tube.
- Centrifuge the cells at 180 x g for 7 minutes. Observe the cell pellet.
- Remove the supernatant from the tube, being careful not to dislodge the cell pellet.
- Resuspend the cell pellet in 4 mL supplemented medium. Pipette the cells up and down with a 10 mL pipette to ensure a homogeneous cell suspension.
- Use a hemacytometer to determine the number of viable cells/mL.
- Dilute the cells in supplemented medium and seed new culture vessels at  $2.5 \times 10^3$  viable cells/cm<sup>2</sup>.
- Incubate the cultures in a 37°C, 5% CO<sub>2</sub>/95% air, humidified cell culture incubator.

**Note:** Damage to cultured HMEC can occur during trypsinization. This damage may result from exposure of the cells to the Trypsin/EDTA solution for excessive lengths of time and/or excessive mechanical agitation. HMEC cultures can alternatively be trypsinized at room temperature incubation for 10-12 minutes. Monitor cell detachment and otherwise follow "Subculture of HMEC" above. TrypLE™ Express (Cat. No.12604) may be substituted for Trypsin/EDTA without affecting HMEC performance. Penicillin/Streptomycin (Cat. No.15140), and Gentamicin/Amphotericin (Cat. No.R-015-10) reagents have been performance validated for routine use and subculture with HMEC.

### Related Products

Human Mammary Epithelial Cells (HMEC) (A10565)  
 Gentamicin/Amphotericin B (10-pack) (R-015-10)  
 Trypsin/EDTA (1X), liquid (R-001-100)  
 Trypsin Neutralizer (1X), liquid (R-002-100)  
 Trypan Blue Stain (15250)

### Contacts

For further information on this or other GIBCO® products, contact Technical Services at the following:

United States TECH-LINE <sup>SM</sup> : 1 800 955 6288  
 Canada TECH-LINE: 1 800 757 8257  
 Europe: eurotech@invitrogen.com

Outside the U.S. and Canada, refer to the GIBCO products catalog for the TECH-LINE in your region.

You may also contact your Invitrogen Sales Representative or our World Wide Web site at [www.invitrogen.com](http://www.invitrogen.com).

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