Working with Human Mammary Epithelial Cells (HMEC)

Introduction

Human mammary epithelial cells (HMEC, Invitrogen Cat. no. A10565) are isolated from normal, reduction mammoplasty tissue and cryopreserved at the end of the 6th passage. Invitrogen offers a validated system for researchers which pairs cells with serum-free HuMEC Ready Medium (Invitrogen Cat. no. 12752-010) for superior growth performance. Applications for HMEC include basic research, drug discovery, cancer research, and development of model systems. Each lot of HMEC is performance tested and characterized by immunocytochemical techniques.

3D culture

3D culture provides a more physiologically relevant cellular environment. Geltrex™ Reduced Growth Factor Basement Membrane Matrix (Invitrogen Cat. no. 12760-021) is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumors that can be used for 3D culture of primary HMEC. Basement membranes are continuous thin sheets of specialized extracellular matrix that separate epithelial and endothelial cells from underlying stroma and play important roles in cellular homeostatsis and tissue architecture. Recent reports have demonstrated the value of 3D culture for modeling malignant and nonmalignant behavior of breast cancer cell lines.[1] Invitrogen's primary HMEC can be cultured in 3D using the Geltrex™ matrix and will form acinar (spherical) structures, typically within 2–4 days. Briefly, HMEC are grown as standard 2D cultures, trypsinized, and seeded as 3D "top" cultures on vessels precoated with Geltrex™. Cells are then incubated in a Geltrex™ solution diluted with HuMEC growth medium. A wide variety of downstream applications using 3D mammary cell cultures have been described, including microscopy (both phase contrast and immunofluorescent detection), and analysis of protein and nucleic acid content. A validated protocol for 3D culture can be found at www.invitrogen.com/mammarycells.

Using Organelle Lights™ and Cellular Lights™ proteins with HMEC

Transient labeling of discrete intracellular regions using targeted fluorescent proteins has been successfully achieved using primary HMEC in combination with Organelle Lights™ and Cellular Lights™ proteins. Organelle Lights™ and Cellular Lights™ proteins are read-to-use fluorescent proteins fused with signal peptides encoded within recombinant baculovirus (BacMam). These products are ideal for the study of dynamic cellular processes which require accurate and specific targeting to subcellular compartments and structures. BacMam delivery of fluorescent proteins into HMEC is very efficient, with >90% of cells expressing the target protein.

The efficient delivery and the genetic content of the Organelle Lights™ marker permits one to transduce a large quantity of primary cells or cell lines in batch mode, aliquot, store frozen, and use as needed, approximating the consistency of stable cell lines without the risk of genetic drift. Fluorescent protein expression is unaffected by the frozen storage. Upon plating, transduced cells can remain brightly fluorescent for more than 120 hours. Specific and precise fluorescence, in combination with the brightness and functional independence of Cellular Lights™ and Organelle Lights™ markers, make them ideal for live-cell applications, including time lapse fluorescent microscopy and high-content screening (HCS). Due to their cellular persistence, Cellular Lights™ and Organelle Lights™ proteins are easy to multiplex with other fluorescent proteins, organic fluorescent dyes, or Qdor®conjugates. They are also compatible with fixation and subsequent immunocytochemical processes. A validated protocol for BacMam transduction (delivery) into HMEC can be found at www.invitrogen.com/mammarycells.

Transfection using Lipofectamine™ LTX Reagent

Lipofectamine™ LTX Reagent offers a new, advanced solution for gene expression studies in hard-to-transfect cells. No other plasmid DNA–specific transfection reagent can match the efficiency, convenience, and gentleness of Lipofectamine™ LTX Reagent. Now combined with a simple and streamlined protocol, there is no need to remove transfection complexes or change /add medium following transfection. You'll spend less hands-on time, and get results faster. A validated protocol for transient transfection of HMEC is available at www.invitrogen.com/mammarycells.

Immunocytochemistry (ICC)

Each lot of HMEC is tested for epithelial-specific markers using fluorescence-based immunocytochemistry. Briefly, adherent cells are fixed and incubated with primary antibodies against cytokeratins (CK) 5/6, 8, 18, and E-cadherin. Secondary antibodies conjugated with Alexa Fluor® 488 dye are used for visualization by fluorescence microscopy. A validated protocol for ICC can be found at www.invitrogen.com/mammarycells.

HMEC FAQs

Are HMEC "normal" cells?

Yes. HMEC are isolated from normal, human reduction mammoplasty tissue.

How many passages/population doublings can I expect from HMEC?

HMEC are guaranteed for a minimum of 16 population doublings after thaw.

What are typical doubling times for HMEC?

HMEC usually have population doubling times of 24–30 hr during the guaranteed lifespan.

How does passage number correlate with population doublings?

One passage is equivalent to ~4 population doublings when using the recommended subculture conditions.

Where can I find technical protocols for HMEC?

Procedures for 3D culture, BacMam gene delivery, transfection, as well as with immunocytochemical staining (ICC), can be found at www.invitrogen.com/mammarycells.

What is the difference between M171 and HuMEC growth media?

Both media are designed for culture of HMEC. However, in-house studies show HuMEC Ready Medium (Invitrogen Cat. no. 12752-010) outperforms M171/MEGS (Invitrogen Cat. no. M-171-500/S-015-5) in both growth rate and lifespan measurements of HMEC. There is no difference between media when staining for cell-specific markers using indirect ICC.

How many T-25 flasks can I seed from 1 vial of HMEC?

One vial (500,000 viable cells) can seed eight T-25 flasks at 2,500 viable cells per cm^2. A hemocytometer should be used to accurately count cells and to ensure an appropriate seeding density.

Is it difficult to culture/passage HMEC?

No! HMEC cultures are easily initiated at 2.5 x 10^3 cells per cm^2 in standard tissue culture plasticware and passaged using a trypsin/EDTA solution.

Reference

1. Kenny P.A., et al. (2007) Morphologies of Breast Cancer Cell Lines in Three-dimensional Assays Correlate with Profiles of Gene Expression. *Molecular Oncology* 1: 84-96.