The base medium for this cell line is Eagle’s Minimum Essential Medium (EMEM; ATCC 30-2003). To make the complete medium, add the following components to the base medium at the indicated final concentrations:
- 10% Fetal Bovine Serum (FBS; ATCC 30-2020)
- 0.01 mg/mL human recombinant insulin (Thermo Fisher cat# 12585014)
- 10 µg/mL Blasticidin S HCl (Gibco cat# A11139-03)

Citation of Strain
If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: MDA-MB-231 VIM RFP (ATCC® HTB-26MET™)

SAFETY PRECAUTION
ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & Storage Instructions
1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Handling Procedure for Frozen Cells
To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor, under strict aseptic conditions.

Handling Procedure for Flask Cultures
The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination.
If the cells are still attached, aseptically remove all but 5 to 10 mL of the shipping medium. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.

If the cells are not attached, add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C.

Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of the base medium at the indicated final concentrations:
- 10% Fetal Bovine Serum (FBS; ATCC 30-2020)
- 0.01 mg/mL human recombinant insulin (Thermo Fisher cat# 12585014)
- 10 μg/mL Blasticidin S HCl (Gibco cat# A11139-03)

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: MDA-MB-231 VIM RFP (ATCC® HTB-26MET™)

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The base medium for this cell line is Eagle's Minimum Essential Medium (EMEM; ATCC 30-2003). To make the complete medium, add the following components to the base medium at the indicated final concentrations:
- 10% Fetal Bovine Serum (FBS; ATCC 30-2020)
- 0.01 mg/mL human recombinant insulin (Thermo Fisher cat# 12585014)
- 10 μg/mL Blasticidin S HCl (Gibco cat# A11139-03)

Breast cancer is the most aggressive form of all cancers, with high incidence and mortality rates. Although epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET) have been implicated in the incidence of cancer metastasis and drug resistance, their impact in cancer progression and patient survival is not fully understood (NIETO et al. 2016). During EMT, epithelial cells lose their polarity, as well as their cell-cell adhesions, and acquire the motile and invasive characteristics of mesenchymal cells (HAY 1995). Proteins such as vimentin (VIM) intermediate filament (IF) are generally upregulated when the cell is in the mesenchymal relative to the epithelial status (GILLES et al. 1999; THIERY and SLEEMAN 2006; RICHARDSON et al. 2012; LAMOUILLE et al. 2014).

The VIM RFP reporter cell line (ATCC HTB-26MET) was created using CRISPR/Cas9 gene editing and the parental MDA-MB-231 breast adenocarcinoma cell line (ATCC HTB-26). HTB-26MET harbors a C-terminal red fluorescent protein (RFP) tag on the vimentin gene. This enables the tracking of the EMT status of cells in vitro by monitoring RFP expression. The integrity of the VIM RFP knock-in has been verified at the genomic, mRNA, and protein level for sequence and expression. Functional evaluation of HTB-26MET shows sensitivity to metastatic breast cancer drugs axitinib (tyrosine kinase inhibitor) and U0126 (MEK1/2 inhibitor) via the inhibition of the inherent signaling pathways which impact EMT.

Subculturing Procedure

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Medium Renewal: 2 to 3 times per week

Comments

Breast cancer is the most aggressive form of all cancers, with high incidence and mortality rates. Although epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET) have been implicated in the incidence of cancer metastasis and drug resistance, their impact in cancer progression and patient survival is not fully understood (NIETO et al. 2016). During EMT, epithelial cells lose their polarity, as well as their cell-cell adhesions, and acquire the motile and invasive characteristics of mesenchymal cells (HAY 1995). Proteins such as vimentin (VIM) intermediate filament (IF) are generally upregulated when the cell is in the mesenchymal relative to the epithelial status (GILLES et al. 1999; THIERY and SLEEMAN 2006; RICHARDSON et al. 2012; LAMOUILLE et al. 2014).

The VIM RFP reporter cell line (ATCC HTB-26MET) was created using CRISPR/Cas9 gene editing and the parental MDA-MB-231 breast adenocarcinoma cell line (ATCC HTB-26). HTB-26MET harbors a C-terminal red fluorescent protein (RFP) tag on the vimentin gene. This enables the tracking of the EMT status of cells in vitro by monitoring RFP expression. The integrity of the VIM RFP knock-in has been verified at the genomic, mRNA, and protein level for sequence and expression. Functional evaluation of HTB-26MET shows sensitivity to metastatic breast cancer drugs axitinib (tyrosine kinase inhibitor) and U0126 (MEK1/2 inhibitor) via the inhibition of the inherent signaling pathways which impact EMT.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty
ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans. While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

Additional information on this culture is available on the ATCC web site at www.atcc.org.

© ATCC 2019. All rights reserved. ATCC is a registered trademark of the American Type Culture Collection. [01/28]