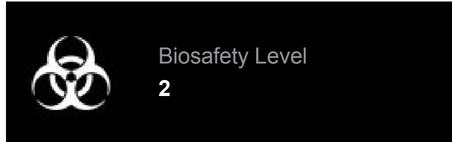
Product Sheet

BT-474 ECAD-EmGFP (ATCC® HTB-20EMT™)

Please read this FIRST



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 Dulbecco's Modified Eagle's Medium (DMEM; ATCC 30-2002). To make the complete medium add the following component:

- 10% Fetal Bovine Serum (FBS; ATCC 30-2020)

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: BT-474 ECAD-EmGFP (ATCC® HTB-20EMT™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Homo sapiens*, human
Tissue: mammary gland, breast, duct; derived from a solid, invasive ductal carcinoma
Disease: ductal carcinoma of the breast
Age: 60 years
Gender: female
Morphology: epithelial
Growth Properties: adherent
DNA Profile:
Amelogenin: X
CSF1PO: 10,11
D13S317: 11
D16S539: 9,11
D5S818: 11,13
D7S820: 9,12
TH01: 7
TPOX: 8
vWA: 15,16

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

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 submersed 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 phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 250 x *g* for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

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. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for

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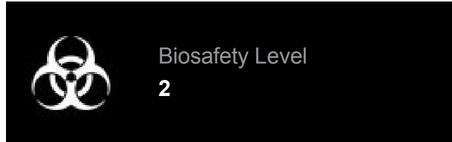

Biosafety Level
2

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any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x *g* for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 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 the 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.
   Cultures can be established between 2 x 10^4 and 2 x 10^5 viable cells/cm^2.
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 2 X 10^4 and 2 X 10^5 cell/cm^2.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week
Note: HTB-20EMT recovers slowly from cryopreservation. It may take two to four weeks for the cells to reach 70-80% confluence in a T-75 flask after thaw. Cells form adherent patches of epithelial-like cells. The patches are compact multilayered colonies that rarely become confluent.

Cryopreservation Medium

Complete growth medium plus 5% (v/v) DMSO (ATCC 4-X)

Comments

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 intermediate filament (IF) proteins 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). Here, we created an ECAD-EmGFP reporter cell line (HTB-20EMT) using the CRISPR/Cas9 gene editing platform and the parental BT-474 breast ductal carcinoma cell line (ATCC HTB-20). The HTB-20EMT cell line harbors a C-terminal green fluorescent protein (EmGFP) tag on the E-cadherin gene. This will enable the tracking of the EMT status of cells in vitro by monitoring GFP expression. The integrity of the ECAD-EmGFP knock-in has been verified at the genomic, mRNA and protein level for sequence and expression. The BT-474 ECAD-EmGFP reporter cell line provides a convenient and sensitive platform for research on the mechanisms of metastasis in vitro and the development of new antitumor drugs for metastatic breast cancer.

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.

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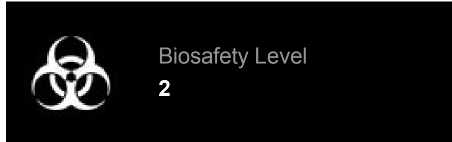

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ATCC Warranty

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

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