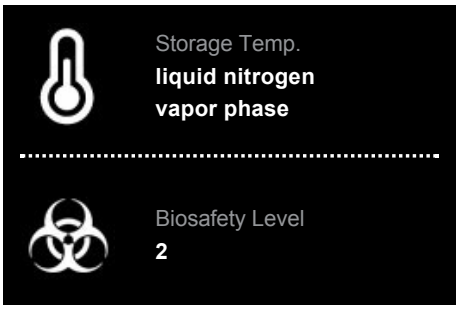
ATCC

Product Sheet

HCT116 VIM RFP (ATCC® CCL-247EMT™)

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

Base growth media for this cell line is McCoy's 5A Medium (ATCC 30-2007). To make the complete medium add the following component to the base medium:

- 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: HCT116 VIM RFP (ATCC® CCL-247EMT™)

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: colon/rectum
Disease: colorectal carcinoma
Age: adult
Gender: male
Morphology: epithelial
Growth Properties: adherent
DNA Profile:
Amelogenin: x,y
CSF1PO: 7,10
D13S317: 10,13
D16S539: 11,13,14
D5S818: 10,11
D7S820: 11,12
TH01: 8,9
TPOX: 8
vWA: 20,22

Note:
The STR profile of the HCT-116 VIM RFP reporter cell line is approximately an 80% match to the parental HCT-116 cell line indicating that the cell lines are related (derived from common ancestry). The STR differences are likely attributed to microsatellite instability in the HT-116 cell line, which is a common feature of this cell line.[1,2,3]

1. Ahmed DP, et al. Epigenetic and genetic features of 24 colon cancer cell lines. Oncogenesis Sep 16;2:e71. doi: 10.1038/oncsis.2013.35, 2013. PubMed: 34042735
2. Gu C, et al. Inositol pyrophosphate profiling of two Hct116 cell lines uncovers variation in Insp8 levels. PLoS One 11(10):e0165286. doi: 10.1371/journal.pone.0165286, 2016. PubMed: 27788189
3. Eshleman JR, et al. Increased mutation rate at the hprt locus accompanies microsatellite instability in colon cancer. Oncogene 10(1):33-7, 1995. PubMed: 7824277

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 125 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

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Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
2

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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 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 4 x 10^4 viable cells/cm^2.
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 2 X 10^4 and 8 X 10^4 cell/cm^2.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week

Cryopreservation Medium

Complete growth medium plus 5% (v/v) DMSO

Comments

The HCT116 VIM RFP reporter cell line, derived from the parental HCT116 (ATCC® CCL-247™) colorectal carcinoma cell line, was created using CRISPR/Cas9 gene editing technology. The HCT116 VIM RFP reporter cell line carries a knock-in red fluorescent protein (RFP) reporter, which was integrated before the stop codon at the last exon of the endogenous vimentin (VIM) gene.

Epithelial to mesenchymal transition (EMT) has been recognized to play an important role in cancer cell metastasis and drug resistance. The EMT pathway is of increasing interest as a novel therapeutic avenue in the treatment of cancer. HCT116 VIM RFP cell line (ATCC® CCL-247EMT™) was created using the CRISPR-Cas9 platform, in which a red fluorescent protein (RFP) reporter was integrated before the stop codon at the last exon of the endogenous vimentin gene, a widely used mesenchymal cell marker. The integrity of the VIM RFP knock-in allele has been verified at genomic, transcriptional, and translational levels. In HCT116 VIM RFP cells, vimentin-RFP expression can be robustly turned on in response to miR-200 family inhibitor treatment, as previously reported (Park et al, Genes Dev, 22: 894-907, 2008). In contrast, the expression of E-cadherin, a marker of epithelial cells, is significantly reduced upon miR-200 inhibitor treatment. In addition, hypomethylating agent 5-Aza-2'-Deoxycytidine treatment can induce an increase in VIM RFP expression effectively. The HCT116 VIM RFP cell line could be a useful in vitro cell model for dissecting the molecular switches underlying EMT and for identifying compounds that target EMT in colorectal cancer.

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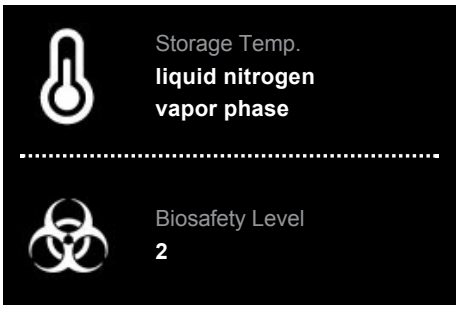

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References

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.
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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.

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