



Product Sheet

B-MEKDD 116 (ATCC® CRL-3069™)

Please read this FIRST



Storage Temp.
**liquid nitrogen
vapor phase**



Biosafety Level
2

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 ATCC-formulated DMEM Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: 10% Bovine Calf Serum and 200 mcg/mL G418

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: B-MEKDD 116 (ATCC® CRL-3069™)

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: *Mus musculus*, mouse
Tissue: breast epithelium (mammary gland)
Cell Type: epithelial cell
Gender: female
Morphology: epithelial-like
Growth Properties: adherent

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 insure 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 $125 \times g$ 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). and dispense into a 25 cm^2 or a 75 cm^2 culture flask. 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).
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 $125 \times g$ 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.



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Subculturing Procedure

Volumes used in this protocol are for 75 cm² flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca⁺⁺/Mg⁺⁺ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 1.0 to 2.0 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. An inoculum of 8 x 10³ to 1.5 x 10⁴ viable cells/cm² is recommended.
6. Incubate cultures at 37°C. Subculture when the cell concentration is between 8 x 10⁴ to 1.5 x 10⁵ cells/cm².

Subcultivation ratio: A subcultivation ratio of 1:6 to 1:12 is recommended.

Medium renewal: Every 2 to 3 days



Cryopreservation Medium

Freeze medium: complete growth medium supplemented with an additional 10% bovine calf serum and 10% DMSO

Storage temperature: liquid nitrogen vapor phase



Comments

B-MEKDD 116 was produced by transfecting parental EpH4, a mouse mammary epithelial cell line, with an expression vector containing Glu-Glu epitope-tagged phosphorylation site MEK1 mutant (MEKDD). After selection in complete medium supplemented with 1 mg/mL G418 for 14 days, individual clones were isolated and expanded.

Activation of MEK1 is mediated through phosphorylation of Ser218 and Ser222 by members of the Raf family of kinases.

This cell line stably expresses constitutively activated form of MEK1 (Asp218/Asp222 MEK1 phosphorylation site mutant), and can be used in MEK-MAPK pathway studies. This cell line is tumorigenic, and can be used to build a breast cancer *in vivo* model. There are four additional related cell lines: EpH 1424 cell line (ATCC CRL-3071), EpH4 1424.1 cell line (ATCC CRL-3209), EpH4 1424.2 cell line (ATCC CRL-3210), and EpH4-Ev cell line (ATCC CRL-3063).

The B-MEKDD 116 cell line produces the constitutively activated MEK1 mutant MEKDD which has been tagged with Glu-Glu, verified at ATCC.



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

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Disclaimers

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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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