ATCC

Product Sheet

# PA317 cyclin E-L (ATCC® CRL-2187™)

**Please read this FIRST**

Biosafety Level
**1**

**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**

Dulbecco's modified Eagle's medium with 4.5 g/L glucose containing 0.1 mg/ml G-418, 90%; calf serum, 10%

**Citation of Strain**

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: PA317 cyclin E-L (ATCC® CRL-2187™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
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800.638.6597 or 703.365.2700
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*Or contact your local distributor*



## Description

**Organism:** *Mus musculus*, mouse
**Strain:** NIH/Swiss
**Disease:** sarcoma
**Cell Type:** fibroblast
**Age:** embryo
**Morphology:** fibroblast
**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

HANDLING PROCEDURE FOR FROZEN CELLS

- Initiate culture as soon as possible upon receipt.

- Thaw by rapid agitation in 37°C water bath. Thawing should be rapid (within 40-60 seconds). As soon as the ice is melted, remove the ampule from the water bath. All of the operations from this point on should be carried out under strict aseptic conditions.

- Transfer the cell suspension and dilute it with the recommended culture medium in a culture flask (see specific batch information above for dilution ratio); incubate at 37°C with 10% $CO_2$ in air atmosphere. Since it is important to avoid excessive alkalinity of the medium during recovery of the cells, it is suggested that the culture medium be placed into the culture flask, tube, etc. and the pH be adjusted, as necessary, prior to the addition of the vial contents. Note that the bicarbonate content of the culture medium will determine whether an atmosphere containing $CO_2$ will be required.

- It is not necessary to remove the freezing additive. However, if desired, the culture medium may be changed to remove the protective freezing additive (dimethylsulfoxide) 24 hours after thawing. If it is desired that the freezing additive be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the above diluted suspension at approximately 125 x g for 10 minutes, discard the fluid and resuspend the cells with growth medium at the dilution ratio given in the specific batch information above.

FLUID RENEWAL
2 times weekly.

SUBCULTURE PROCEDURE
Remove medium, rinse with trypsin (0.25%) - EDTA (0.03%) solution. Add 1-2 ml of fresh trypsin solution and allow flasks to remain at room temperature until cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.
Subcultivation ratio: 1:6 to 1:10 is recommended.

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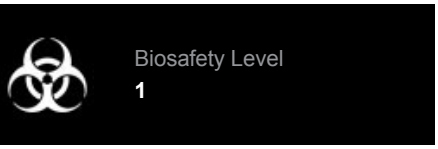


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## Handling Procedure for Flask Cultures

HANDLING PROCEDURE FOR FLASK CULTURES (MONOLAYER)
The flask was seeded with cells (see specific batch information above for concentration), grown and completely filled with medium to prevent loss of cells in transit. Remove all of the medium (which can be saved and used as fresh medium) except for a sufficient volume (5-10 ml) to cover the floor of the flask. Incubate at 37°C. The shipping medium contains reduced sodium bicarbonate suitable for a 5% $CO_2$ in air incubator. DMEM usually contains 3.7 grams of sodium bicarbonate per liter and should be incubated in a 10% $CO_2$ in air atmosphere. Sometimes in transit the cultures are handled roughly and most of the cells become detached and float in the culture medium. If this has occurred remove the entire contents of the flask and centrifuge at 300 x g for 15 minutes. Draw off the excess supernatant medium, resuspend the cells in 10 ml of the culture medium and plant the entire cell suspension in a single flask of suitable size (about 25 sq. cm.).

## Subculturing Procedure

**Subcultivation Ratio:** A subcultivation ratio of 1:6 to 1:10 is recommended
**Medium Renewal:** Twice per week
Remove medium, rinse flask with fresh 0.25% trypsin, 0.02% EDTA and allow the flask to sit at room temperature until the cells detach. Add fresh medium, aspirate and dispense into new flasks.

## Comments

PA317 cyclin E-L is an amphotropic retrovirus producing cell line constructed from the PA317 (see ATCC CRL-9078) fibroblast cell line by introduction of a retrovirus expression vector (LXSN).
The LXSN vector uses the 5'long terminal repeat of Moloney murine sarcoma virus to express the inserted cDNA and contains the neomycin phosphotransferase gene (G418 resistance) as a selectable marker.
The full length cDNA coding human cyclin E-L (1.7 kb HindIII fragment) was treated with Klenow fragment and cloned into the HpaI site of the vector.
The PA317 cyclin E-L cell line expresses human cyclin E-L.
Cyclin E is a nuclear protein essential for the G1 to S phase transition.
Mammalian cells express two forms of cyclin E protein which differ from each other by the presence (cyclin E-L) or absence (cyclin E-S) of a 15 amino acid amino terminal domain.
Fibroblasts engineered to constitutively overexpress either form of cyclin E show elevated cyclin E dependent kinase activity and a shortened G1 phase of the cell cycle.

## References

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

## Biosafety Level: 1

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

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. 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 strain. 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

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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 strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

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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