



Product Sheet

Lec8 [originally named Pro-5WgaRVIII3D] (ATCC® CRL-1737™)

Please read this FIRST



Storage Temp.
**liquid nitrogen
vapor phase**



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

Alpha minimum essential medium, 90%, (GIBCO catalog #A10490-01); fetal bovine 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: Lec8 [originally named Pro-5WgaRVIII3D] (ATCC® CRL-1737™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
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800.638.6597 or 703.365.2700
Fax: 703.365.2750
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Or contact your local distributor

Description

Organism: *Cricetulus griseus*, hamster, Chinese

Tissue: ovary

Gender: female

Morphology: epithelial

Growth Properties: monolayer

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 x g for 5 to 10 minutes.
4. Resuspend the cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm² or a 75 cm² 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% CO₂ in air atmosphere is recommended if using the medium described on this product.

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.

1. Remove and discard culture medium.
2. 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 10 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.
3. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
4. Add appropriate aliquots of the cell suspension to new culture vessels.
5. Incubate cultures at 37°C.

ALTERNATIVELY, cultures also can be grown as a suspension and maintained by addition or replacement of fresh medium. Start cultures at 2 x 10⁴ cells/mL and maintain between 2 x 10⁴ and 1 x 10⁶ cells/mL.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended

Medium Renewal: 2 to 3 times per week



Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in Culture Of Animal Cells: A Manual Of Basic Technique by R. Ian Freshney, 5th edition, published by Wiley-Liss,



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N.Y., 2005.



Cryopreservation Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.



Comments

The cells exhibit a drastic reduction in the transport of UDP-galactose into the Golgi compartment.

The cells are proline auxotrophs and must be grown in a medium that contains proline (40 mg/L).

The population contains Pro⁺ revertants at high frequency (approximately 1 in 250 cells).

These cells may also be grown as a suspension culture if agitated. Keep the cell concentration between 2×10^4 and 1×10^6 cells/mL.



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.

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

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