ATCC

Product Sheet

Pneumocystis carinii (ATCC® PRA-159™)

Please read this FIRST

Storage Temp.
Frozen: -70°C or colder
Freeze-Dried: 2°C to 8°C
Live Culture: See Protocols Section

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.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Pneumocystis carinii* (ATCC® PRA-159™)

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

Strain Designation: M167-6
Depositor: MT Cushion
Isolation: Isolated by M Collins from the lungs of an immunosuppressed Lewis male rat (Rattus norvegicus), Cincinnati, OH, October 2003.

Propagation

Growth Conditions
in-vivo cultivation, rat

Protocols

Storage and Culture Initiation
Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place it in a 35°C water bath , until thawed (2-3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate an uninfected, immunosuppressed rat. Follow the protocol for maintenance *in vivo*. The course of infection may be longer or shorter than usual depending on percent recovery of the parasite from the frozen state.

Culture Maintenance
When the frozen ampule arrives, store it as indicated above until ready to use. The following directions for establishing an infection are adapted from: Boylan, C.J. and W.L. Current. 1992. Improved rat model of *Pneumocystis carinii pneumonia*: induced laboratory infections in *Pneumocystis*-free animals. Infect. Immun. 60: 1589-1597. They must be followed carefully to assure success.

1. Seven days prior to thawing the frozen ampule, immunosuppress rats (viral antibody-negative male or female rats weighing 120-140 grams each, any of several strains, e.g., Lewis, Sprague-Dawley, Fischer 344) by administering one injection of 4 mg of methylprednisolone acetate (Upjohn Co., Kalamazoo, MI).
2. On day seven, thaw the frozen ampule rapidly in a 35°C water bath as indicated above.
3. Transfer the thawed contents to a centrifuge tube and add an equal volume of RPMI 1640 medium (GIBCO 31800-022) containing 20% (v/v) heat-inactivated fetal bovine serum.
4. Centrifuge at 1000 x g for 5 minutes, remove supernatant and resuspend the pellet with medium specified in step 3 to a volume such that the final concentration of the *P. carinii* nuclei is 10^7-10^8 per mL (the concentration of the nuclei will be specified on the certificate of analysis shipped with the frozen ampule).
5. Aspirate 0.1 mL of the well-mixed suspension into a 1.0 mL syringe fitted with a three-inch, 20-gauge curved stainless steel animal feeding tube (Popper and Sons, New Hyde Park, NY). Keep filled syringe on ice until ready to inoculate.
6. Lightly anesthetize rats by exposing them briefly to halothane.
7. Suspend anesthetized rats by their upper incisors on a wire loop at the top of a board held at a 60 degree incline. Pull tongue to one side of the lower incisors with a pair of forceps, insert the feeding tube prepared in step 5 and express 0.1 mL of inoculum followed by 0.4 mL of air into the trachea. Note: To assure correct placement of the inoculum into the trachea, direct the feeding tube along the back of the tongue into the larynx while palpating the trachea.
8. Continue weekly injections of 4 mg of methylprednisolone into inoculated rats.
9. Six weeks post-inoculation, check for infection by killing a rat, removing the lungs and preparing impression smears.

Cryopreservation

No protocol available at this time.

References

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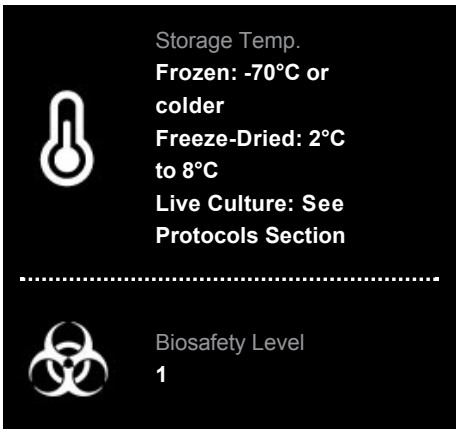

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References and other information relating to this product are available online at www.atcc.org.

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.

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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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