ATCC

Product Sheet

Pneumocystis murina (ATCC® PRA-111™)

Please read this FIRST

Storage Temp.

Frozen Cultures:
-70°C for 1 week; liquid N_2 vapor for long term storage

Freeze-dried Cultures:
2-8°C

Live Cultures:
See Protocols section for handling information

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 murina* (ATCC® PRA-111™)

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

Depositor: MT Cushion
Isolation: Not available

Propagation

Growth Conditions
Culture System: *in-vivo* cultivation, mouse

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 ampoules 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 such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed.
2. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate an uninfected, immunosuppressed mouse. 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 have been adapted and modified 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. Up to two weeks* prior to thawing the frozen ampule, immunosuppress mice (viral antibody-negative male or female mice, any strain, e.g., C57, ICR, BALB/c) by administering dexamethasone at a concentraion of 4 mg/liter to their drinking water. (IVX Animal Health, Inc., St. Joseph, MO).
   ***Note:** Immunosuppression of mice may alternatively begin on the same day as the first inoculation with *Pneumocystis*.
2. 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. murina* nuclei is 10^7 to 10^8 per mL (the concentration of the nuclei will be specified on the certificate of analysis shipped with the frozen ampule).
5. Aspirate 0.05 mL of the well-mixed suspension into a 1.0 mL syringe (optionally fitted with a 20-gauge stainless steel animal feeding tube; Popper and Sons, New Hyde Park, NY). Keep filled syringe on ice until ready to inoculate.
6. Lightly anesthetize mice by exposing them briefly to isoflurane.
7. Perform intranasal inoculation using the syringe prepared in step 5 by expressing 0.05 mL of inoculum onto the nares. Since mice are obligate nose-breathers, the *Pneumocystis* suspension will immediately be inhaled.
8. Continue administration of dexamethasone to inoculated mice as indicated above.
9. Six weeks post-inoculation, check for infection by killing a mouse, removing the lungs and preparing impression smears.

Cryopreservation

No protocol available at this time.

References

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

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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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