



Pneumocystis murina

PRA-111™

Description

Pneumocystis murina has applications in opportunistic pathogen research. This parasitic protozoan is cultivated in mice.

Type strain: No

Storage Conditions

Product format: Frozen

Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always

used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Host:

In vivo cultivation, mouse

Handling Procedures

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 concentration 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.
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Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Pneumocystis murina* (ATCC PRA-111)

References

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

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