Sarcocystis neurona

PRA-420™

Description

Strain designation: SN3.E1

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 2

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: BT (ATCC CRL-1390)
Instructions for complete medium: Media: Advanced Minimum Essential medium (MEM) (Gibco Cat # 12492-013) supplemented with 4% fetal bovine serum (ATCC® cat. 30-2020).

Alternative media: ATCC® 30-2002 [Dulbecco's
Temperature: 37°C
Atmosphere: 95% Air, 5% CO_2

Handling Procedures

Cell Line Maintenance

1. To establish a cell culture from the frozen state, place an ampule of ATCC® CRL-1390™ in a water bath set at 35°C for 2-3 min. Immerse the vial just sufficient to cover the frozen material. Do not agitate the vial.

2. Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 10.0 mL of fresh Advanced MEM with 4% (v/v) heat-inactivated fetal bovine serum (HIFBS)* in a T-25 tissue culture flask.
3. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.
4. Incubate in a 37°C CO_2 incubator with the caps screwed on tightly.
5. Change the medium 1-2 times per week.

   *Fetal bovine serum is available from ATCC (catalog number 30-2020). Serum is heat-inactivated by exposure to 56°C for 30 minutes. This treatment will inactivate proteins of the complement pathway. Remove the serum from the refrigerator and aseptically distribute in 100 mL aliquots to sterile 125 mL screw-capped bottles. Immerse bottles in a 35°C water bath for 5 minutes. Do not directly transfer bottles from the refrigerator to 56°C. Transfer the bottles to a 56°C water bath and begin timing for 30 minutes. To avoid contamination, do not allow the level of the water in the bath to come in contact with the lip of the screw cap. It is best to leave one inch between the serum level in the bottle and the lip of the cap and to fill the water bath to a level just slightly above the level of the serum. To assure even heating of the serum, swirl the bottle(s) every ten minutes. **Note:** Some suppliers provide serum already heat-inactivated.

Transferring the Cell Line

1. When the cell line forms a confluent layer, remove all the medium and replace it with 2 mL of 0.25% (w/v) trypsin dissolved in Hank's Balanced Salt Solution.
2. Gently distribute the trypsin over the monolayer, remove the trypsin, and place the flask at 37°C for 5 min.
3. Add 2 mL of Advanced MEM with 4% (v/v) HIFBS in a T-25 tissue culture flask and detach any cells still adherent by alternately aspirating the medium into a pipette and discharging the contents over the monolayer.
4. Distribute the cell suspension in 0.5 mL aliquots to four T-25 flasks containing 10 mL of fresh culture media.
5. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.
6. Incubate in a 37°C CO_2 incubator with the caps screwed on tightly.

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 transfer contents to a T-25 tissue culture flask containing a fresh monolayer of ATCC® CRL-1390™ cells and 10 mL of Advanced MEM with 4% (v/v) HIFBS.
3. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.
4. Incubate in a 37°C CO_2 incubator with the caps screwed on tightly. Observe the culture daily under an inverted microscope for the presence of schizont and merozoite stages of *Sarcocystis*.

Culture maintenance:

1. Remove the medium from a fresh confluent monolayer of ATCC® CRL-1390™ cells in a T-25 tissue culture flask and replace it with 10 mL of Advanced MEM with 4% (v/v) HIFBS.
2. To transfer the *Sarcocystis* culture, remove the medium containing free merozoites and centrifuge at 1300 x g for 10 min.
3. Remove the supernatant and resuspend the parasite pellet in 1 mL of culture medium. Transfer the resuspended pellet to the fresh flask of CRL-1390™ cells.
4. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.
5. Incubate in a 37°C CO_2 incubator with the caps screwed on tightly.

Cryopreservation:

1. Harvest the *Sarcocystis* culture by gently agitating the contents of each flask. Transfer all but approximately 1 mL of the culture medium to 15 mL plastic centrifuge tubes. Detach the remaining infected and uninfected tissue culture cells by scraping the inner surface of the flask with a cell scraper. Pass the resulting cell suspension through a syringe equipped with a 27-gauge ½-inch needle and pool this suspension with the culture fluid.
2. Spin the cell suspensions at approximately 50 x g for 3 min, to remove the cellular debris.
3. Transfer the supernatants to new 15 mL plastic centrifuge tubes. Centrifuge at 1300 x g for 10 min.
4. Pool the parasite pellets and adjust the concentration to ~2.0 x 10^7

merozoites/mL with fresh Advanced MEM.
*If the concentration is too low, centrifuge at 1300 x g for 10 min and resuspend in the volume of Advanced MEM required to yield the desired parasite concentration.

5. Mix equal volumes of parasite suspension and fresh medium containing 20% DMSO and 50% HIFBS to yield a final concentration of ~1 x 10^7 merozoites/mL in 10% DMSO, 25% HIFBS. The time from the mixing of the parasite preparation and the cryoprotective solution before the freezing process begins should be no less than 15 min and no more than 30 min.
6. Dispense in 0.5 mL aliquots to 1.0-2.0 mL sterile plastic screw-capped cryovials.
7. Place the vials in a controlled rate freezing unit. From room temperature, cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. The cooling rate in this apparatus is approximately -1°C/min.
8. Store in either the vapor or liquid phase of a nitrogen refrigerator.
9. 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 thawing.
10. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing a fresh monolayer of ATCC® CRL-1390™ cells and 10 mL of Advanced MEM with 4% (v/v) HIFBS.
11. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.
12. Incubate in a 35-37°C CO_2 incubator with the caps screwed on tightly.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Sarcocystis neurona* (ATCC PRA-420)

References

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

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