ATCC

Product Sheet

D2N (ATCC® TIB-58™)

Please read this FIRST

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

RPMI 1640 medium with 0.01 mM 2-mercaptoethanol, 90%; heat- inactivated 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: D2N (ATCC® TIB-58™)

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

Organism: *Mus musculus*, mouse
Strain: DBA/2
Tissue: spleen
Disease: leukemia
Morphology: lymphoblast
Growth Properties: suspension

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

HANDLING PROCEDURE FOR FROZEN CELLS

- Initiate culture as soon as possible upon receipt.

- Thaw by rapid agitation in 37°C water bath. Thawing should be rapid (within 40-60 seconds). As soon as the ice is melted, remove the ampule from the water bath and immerse in 70% ethanol at room temperature. All of the operations from this point on should be carried out under strict aseptic conditions.

- The cells are supplied in two different types of glass ampules. One is a standard ampule, the neck of which must be scored with a sharp file that has been immersed in ethanol. A definitive sharp nick about 1/8" in length on one side is necessary. The second type is prescored and is identifiable by a gold band around the ampule neck, and should not be scored with a file.

- Break the neck of the ampule between several folds of a sterile towel.

- Transfer the cell suspension and dilute it with the recommended culture medium in a culture flask (see specific batch information above for dilution ratio); incubate at 37°C with 5% CO_2 in air atmosphere. Since it is important to avoid excessive alkalinity of the medium during recovery of the cells, it is suggested that the culture medium be placed into the culture flask, tube, etc. and the pH be adjusted, as necessary, prior to the addition of the ampule contents. Note that the bicarbonate content of the culture medium will determine whether an atmosphere containing CO_2 will be required.

- It is not necessary to remove the freezing additive. However, if desired, the culture medium may be changed to remove the protective freezing additive (dimethylsulfoxide) 24 hours after thawing. If it is desired that the freezing additive be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the above diluted suspension at approximately 125 x g for 10 minutes, discard the fluid and resuspend the cells with growth medium at the dilution ratio given in the specific batch information above.

SUBCULTURE PROCEDURE

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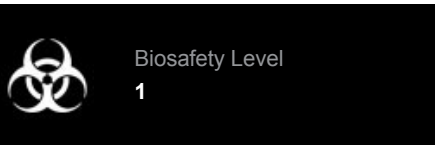

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CHESEBRO PROTOCOL FOR GROWTH OF MOUSE LEUKEMIA LINES

1) MEDIUM RPMI 1640 with 200 units/ml penicillin. Before use add 10% heat-inactivated fetal bovine serum and also 2-mercaptoethanol to a final concentration of 10(-5) molar. (A stock of 0.01 M 2-ME solution in PBS which is diluted 1:1000 into the tissue culture medium. The 2-ME does not need to be fresh; and the stock solution has been used for 1-2 years.) Most, but not all, of the mouse leukemia lines grow only in medium with 2-ME. MEM or McCoy's have not been substituted for 1640 but may work. Most lines do not grow as well in HEPES-containing medium.

2) Cells are grown in stationary suspension cultures at 37°C in 5% CO_2-95% air. Use 25 cm^2 flasks containing 7 ml medium. Cells received after shipping in tissue culture flasks full of medium should be pelleted by low speed centrifugation, resuspended in fresh medium, and viable cells counted. Initially, new cultures should be started at several different cell concentrations (i.e. 3 x 10(4), 1 x 10(5), 3 x 10(5), 1 x 10(6) cells/ml) to insure successful growth. This is particularly important if there is a high proportion of dead cells in the population. Cells can also be started by I.V. or I.P. injection of appropriate mouse strains (see #4 and #6 below).

3) For routine passage 1-7 x 10(5) cells from a fully grown-out culture are seeded into a fresh flask containing 7 ml medium. Most lines grow to a maximum final concentration of 1-4 x 10(6)/ml, it is usually not necessary to count cells, but examine the cultures to see that the cells are in good condition and present in high concentrations. Then 0.05 ml and 0.03 ml of suspension culture are passed to two new flasks. The higher seeding inoculum usually grows out to maximum concentration in 2-3 days, and the lower one in 4-5 days. Under an inverted microscope at 400X, the viable-growing cells appear to have a sharp even change in refractive index at the plasma membrane, and many doublet cells are present. When the cells achieve their maximum concentration, they begin to die off, the dead cells having a coarse granular appearance, the nuclear appears obvious and clumped, the plasma membrane often jagged. The ratio of dead to live cells increases rapidly in the next 1-2 days. Cultures should be passed before the number of dead cells begins to increase. However, in emergency situations, cultures with as few as 1% viable cells have been passaged and saved.

4) Cells derived from ascites passaged lines can be passed in vivo in appropriate mouse strains. Inject 0.5-5 x 10(6) cells I.P., however in few cases where this has been checked, as few as 10(3) cells grew out reliably. Mice may or may not get grossly enlarged abdomens. Most lines, mice will die in 10-14 days. When unfamiliar with a particular line, cells should be harvested from the peritoneal cavity as soon as any abdominal enlargement is noted; since, with many lines, the transition from slight enlargement to death can occur in 24 hours. Typical yield is about 500 x 10(6) cells/mouse.

5) All cell lines from both in vivo and in vitro sources have been frozen successfully using standard techniques in medium plus 10% DMSO at concentrations ranging from 1-50 x 10(6) cells/ml. (The higher the better in terms of recovering more viable cells after thawing.) The FV lines which have never been adapted to in vivo growth outside the lymphoid system are the most difficult to freeze successfully. For these, freeze at a minimum concentration of 10-15 x 10(6) cells/ml. Under these conditions, some batches work and some do not.

6) Applicable to BB88 cells only (see TIB 55). Lines derived from splenic or lymph node tumors which have not been adapted to growth outside the lymphoid system can also be grown in vitro. Inject 5-30 x 10(6) cells I.V. Mice are followed for splenic enlargement by palpation under ether anesthesia. Mice with enlarged spleens or large abdominal masses are sacrificed and tumor-containing organs are dissociated in buffer for passage or use.

Handling Procedure for Flask Cultures

HANDLING PROCEDURE FOR FLASK CULTURES (SUSPENSION)

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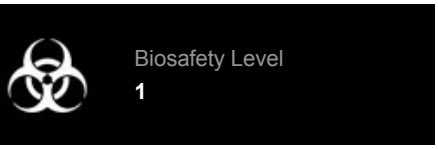

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The flask was seeded with cells (see specific batch information above for concentration), grown and completely filled with medium to prevent loss of cells in transit. Upon receipt incubate the flask in an upright position for several hours to return the flask contents to 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 300 x g for 15 minutes. Resuspend the cell pellet in 10-12 ml of the shipping medium. From this suspension remove a sample for a cell count and viability so that the cell density of the suspension can be adjusted to 2-3 x 10(5) viable cells/ml. If the suspension needs to be diluted use the shipping medium. Incubate the culture in a flat position at 37°C in a 5% CO_2 in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure described above.

Subculturing Procedure

Medium Renewal: Every 2 to 3 days
Cultures can be maintained by addition or replacement of fresh medium.
Start cultures at 2 X 10 exp5 cells/ml and maintain between 1 X 10 exp5 and 1 X 10 exp6 cells/ml.
The cells may also be grown as ascites in syngeneic mice.
Inject 2 X 10 exp5 cell per mouse and harvest as soon as abdominal enlargement is observed (about 10 days).

Comments

This line was derived from a leukemia induced by N-tropic Friend murine leukemia virus.
The cells are negative for surface immunoglobulin (sIg-) and Thy-1.2 (Thy-1.2-).
Erythroid differentiation and hemoglobin synthesis are NOT inducible by DMSO.
Tested and found negative for ectromelia virus (mousepox).

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.

ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. 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 product. 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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