BC16A

TIB-59™

Description

Organism: *Mus musculus*, mouse
Tissue: Spleen
Morphology: lymphoblast
Growth properties: Suspension
Disease: Leukemia

Storage Conditions

Product format: Frozen

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.

Handling Procedures

Unpacking and 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.

Complete medium: RPMI 1640 medium with 0.01 mM 2-mercaptoethanol, 90%; heat-inactivated fetal bovine serum, 10%

Handling Procedure: 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

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.

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).

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: BC16A (ATCC TIB-59)

References

References and other information relating to this material are available at

www.atcc.org.

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