**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**

The base medium for this cell line is ATCC-formulated Dulbecco’s Modified Eagle’s Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. Culture Medium: Dulbecco’s modified Eagle’s medium, 90%; 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: 5E9C11 (ATCC® HB-21™)

**Description**

Organism: *Mus musculus* (B cell); *Mus musculus* (myeloma), mouse (B cell); mouse (myeloma)

Isotype: IgG1; kappa light chain

Disease: Leukemia

Cell Type: hybridoma: B lymphocyte

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**

**Part A. FROZEN CELLS**

Vol./Ampule: 1.0 mL.

Recommended Handling Upon Receipt: Initiate culture as soon as possible upon receipt. Thaw by rapid agitation in 37°C water bath. See instructions on back.

Dilute ampule contents with culture medium (see batch data above). Add fresh medium (depending on cell density) every 2-3 days.

**Handling Procedure for Flask Cultures**

**Part B. FLASK CULTURES**

Recommended Handling Upon Receipt: Suspension Cultures: The culture flask was seeded, see batch data above, 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 2-5 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% CO2 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

This line has carried by passage as ascites in pristane primed (0.5 ml, 7 days prior to injection of cells) mice. The mice are injected with 0.3 to 0.5 ml of cells, and the ascites is collected 7 to 10 days later.

Recent information from the donor indicates that the cells are readily grown in culture using the above medium.

Cultures can be maintained by addition or replacement of fresh medium. Start cultures at 5 x 10 exp4 cells/ml and do not allow the cells to exceed 8 X 10 exp5 cells/ml.

**Comments**
Animals were immunized with HSB-2 cells (ATCC CCL-120.1, an acute lymphocytic T leukemia cell line). Spleen cells were fused with P3X63Ag8 myeloma cells. This line is derived from the same fusion used to produce ATCC HB-2 and ATCC HB-22. The antibody recognizes a 90,000 dalton cell surface antigen (the same as that recognized by OKT 9, ATCC CRL-8021). The antibody precipitates human transferrin receptor. It does not fix complement. Tested and found negative for ectromelia virus (mousepox).

References

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