Description

Organism: Rattus norvegicus (B cell); Mus musculus (myeloma), rat (B cell); mouse (myeloma)
Isotype: IgG2a
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

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. All of the operations from this point on should be carried out under strict aseptic conditions.
- 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% CO₂ 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 vial contents. Note that the bicarbonate content of the culture medium will determine whether an atmosphere containing CO₂ 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.

FLUID RENEWAL
Add fresh medium (as cell density increases) every 2-3 days.

SUBCULTURE PROCEDURE
Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1-2 x 10(5) viable cells/ml. Maintain cell density between 1 x 10(5) and 1 x 10(6) viable cells/ml.

Handling Procedure for Flask Cultures
HANDLING PROCEDURE FOR FLASK CULTURES (SUSPENSION)

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 2-5 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

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.

Comments

Animals were immunized with BMS2 murine bone marrow stromal cells. Spleen cells were fused with Sp2/0-Ag14 myeloma cells. The antibody reacts with mouse CD9 (expressed by bone marrow stromal cells, megakaryocytes, platelets, myeloid cells and subpopulations of mature lymphocytes.

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.

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