

Data Sheet

PAGE 1/3

BCRJ Code:	0016
Cell Line:	3H5-1
Species:	Mus musculus (B cell); Mus musculus (myeloma), mouse (B cell); mouse (myeloma)
Vulgar Name:	Mouse
Cell Type:	Hybridoma: B Lymphocyte
Morphology:	Lymphoblast
Disease:	Dengue
Growth Properties:	Suspension
Derivation:	Spleen cells were fused with P3X63Ag8 myeloma cells.
Tumor Formation::	YES
Products:	immunoglobulin; monoclonal antibody; against a type specific determinant on Dengue virus-2)
Biosafety:	1
Additional Info:	Animals were immunized with dengue virus type 2 antigens from infected mouse brains (the prototype strain New Guinea C was used). The antibody is type specific. Spleen cells were fused with P3X63Ag8 myeloma cells. Antibodies should be prepared as ascites
Culture Medium:	Hybri-Care Medium from ATCC (Catalog No. 46-X), 1.5 g/L sodium bicarbonate and fetal bovine serum to a final concentration of 10%.

Data Sheet

PAGE 2/3

Subculturing:

Cultures can be maintained by addition of fresh medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1×10^5 viable cells/mL. Maintain cultures at a cell concentration between 1×10^5 and 1×10^6 cells/mL. NOTE: Do not allow the cell concentration to exceed 1×10^6 cells/mL.

Subculturing Medium Renewal:

Every 2 to 3 days

Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO₂), 5% Temperature: 37°C

Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:

SAFETY PRECAUTION: It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris.

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions.
3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately $125 \times g$ for 5 to 7 minutes.
4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio).
5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line).

NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).

Data Sheet

PAGE 3/3

References:

Henchal EA, et al. Dengue virus-specific and Flavivirus group determinants identified with monoclonal antibodies by indirect immunofluorescence. Am. J. Trop. Med. Hyg. 31: 830-836, 1982. PubMed: 6285749

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[CVCL D292](#)