

Code:	0268
Cell Line:	D1-4G2-4-15
Species:	Mus musculus (B cell); Mus musculus (myeloma), mouse (B cell); mouse (myeloma) Vulgar Name: Mouse
Tissue:	Spleen
Morphology:	Lymphoblast-Like
Growth Properties:	Suspension
Derivation:	Animals were immunized with dengue virus type 2 antigens. Spleen cells were fused with P3X63Ag8 myeloma cells.
Products:	immunoglobulin; antibody against flavivirus group antigen
Biosafety:	1
Additional info:	The antibody reacts with flavivirus group specific antigens.
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%.
Subculturing:	Cultures can be maintained by addition of fresh medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 10e5 viable cells/mL. Maintain cultures at a cell concentration between 2 x 10e5 and 1 x 10e6cells/mL. Medium Renewal: 2 to 3 times a week. Subcultivation ratio:
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO ₂), 5% Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)

**Thawing Frozen
Cells:**

SAFETY PRECAUTION: It is highly recommended 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 submerged 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.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the Oring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. For cells that are sensitive to DMSO it is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio).
5. Incubate the culture in an appropriate atmosphere and temperature (see "Culture Conditions" for this cell line).

NOTE: It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

References:

922: Henschel 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

Depositors:

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ATCC:

HB-112