

Code:	0227
Cell Line:	Sp2/0-Ag14
Species:	Mus musculus (B cell); Mus musculus (myeloma), mouse (B cell); mouse (myeloma)
	Vulgar Name: Mouse; Balb/C
Tissue:	Spleen
Morphology:	Lymphoblast-Like
Growth Properties:	Suspension
Derivation:	The line was formed by fusing BALB/c spleen cells (from mouse immunized with sheep RBCs) with the P3X63Ag8 myeloma.
Applications:	This cell line is a fusion partner for production of somatic cell hybrids.
Products:	Antigen expression: H-2d
Biosafety:	1
Additional info:	The cells do not secrete immunoglobulin, are resistant to 8-azaguanine at 20 µg/ml and are HAT sensitive.
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate and 10% of fetal bovine serum.
Subculturing:	<p>Cultures can be maintained by the addition of fresh medium or replacement of medium. Maintain cell density between 5 X 10⁴ and 5 X 10⁵ viable cells/mL. Some cells can attach and be transferred by shaking them loose.</p> <p>Medium Renewal: Add fresh medium every 2 to 4 days (depending on cell density)</p> <p>Subcultivation ratio:</p>
Culture	Atmosphere: air, 95%; carbon dioxide (CO ₂), 5% Temperature:

Conditions: 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 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 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:

Ozato K, Sachs DH. Monoclonal antibodies to mouse MHC antigens. III. Hybridoma antibodies reacting to antigens of the H-2b haplotype reveal genetic control of isotype expression. *J. Immunol.* 126: 317-321, 1981. PubMed: 6935293

Shulman M, et al. A better cell line for making hybridomas secreting specific antibodies. *Nature* 276: 269-270, 1978. PubMed: 714156

Goodrum FD, et al. Adenovirus early region 4 34-kilodalton protein directs the nuclear localization of the early region 1B 55-kilodalton protein in primate cells. *J. Virol.* 70: 6323-6335, 1996. PubMed: 8709260

Chen H, et al. Octamer binding factors and their coactivator can activate the murine PU.1 (spi-1) promoter. *J.*

Biol. Chem. 271: 15743-15752, 1996. PubMed: 8663022 Cross
References Nucleotide (GenBank) : NC_001702 Murine type C
retrovirus, complete genome. Nucleotide (GenBank) : X94150
Retroviridae complete genome (murine type C retrovirus).

Depositors: Instituto Oswaldo Cruz, Rio de Janeiro.

ATCC: CRL-1581