

Banco de Células do Rio de Janeiro

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BCRJ Code:	0092
Cell Line:	GK1.5
Species:	Rattus norvegicus (B cell); Mus musculus (myeloma), rat (B cell); mouse (myeloma)
Vulgar Name:	Rat; Mouse, Lewis (B Cell); Balb/C (Myeloma)
Tissue:	Spleen
Cell Type:	Hybridoma: B Lymphocyte
Morphology:	Lymphoblast-Like
Growth Properties:	Suspension
Derivation:	Animals were immunized with the cloned cytotoxic T lymphocyte lines V4 and 243/2.5. Spleen cells were fused with Sp2/0-Ag14 myeloma cells.
Applications:	The antibody profoundly blocks antigen specific murine class II MHC antigen reactive helper T lymphocyte lines.
Products:	immunoglobulin; monoclonal antibody; against mouse helper, inducer T cells (L3T4 antigen, CD4)
Biosafety:	1
Addtional Info:	L3T4 is expressed on mouse helper/inducer T cells and is analogous to the human Leu-3/T4 molecule.
Culture Medium:	Iscove's Modified Dulbecco's Medium (IMDM) with 4500 mg/L glucose 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 1 x 10e5 viable cells/mL. Maintain cultures at a cell concentration between 1 x 10e5 and 1 x 10e6 cells/mL. NOTE: Do not allow the cell concentration to exceed 1 x 10e6 cells/mL. Population Doubling Time about: 24-30 hours

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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 × 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).

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