Designation: **P3X63Ag8.653**

CLS order number: Cryovial: 400118
Vital: 440118

Origin and General Characteristics	
Organism:	Mus musculus (mouse)
Strain:	BALB/c
Tissue:	Plasmacytoma; B lymphoblast
Morphology:	Lymphoblast
Cell type:	Myeloma
Growth Properties:	Suspension/adherent
Description:	The cells are resistant to 8-azaguanine and are HAT sensitive. They can be used as fusion partners for producing hybridomas. The cells do not secrete immunoglobulin. The cells have been reported to be cholesterol auxotroph due to a deficiency in 3-ketosteroid reductase activity.
References:	Kearney JF et al. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J Immunol 123: 1548-50, 1979.
Culture Conditions and Handling	
Culture Medium:	RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% fetal bovine serum (MG-70, CLS order number 820700).
Subculturing:	Subculture by collecting any floating cells in a centrifuge tube. Rinse the adherent cells using PBS without calcium and magnesium (3-5 ml PBS for T25, 5-10ml for T75 cell culture flasks). Add TrypleExpress (1-2ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at 37°C for 10 minutes. Combine all cells, and start new cultures at 4 x 10^5 cells/ml. The cell density should not exceed 2 x 10^6 cells/ml.
Seeding density:	1 – 2 x 10^4/cm^2
Fluid Renewal:	Every 3 to 4 days; collect floating cells, centrifuge and add to the flask together with fresh medium.
Freeze Medium:	CM-1 (CLS order number: 800125, 25ml, 800150, 50ml)
Freezing recovery:	After thawing, plate the cells at 5 x 10^4 cells/cm^2 and allow the cells to recover from the freezing process and to adhere for at least 24 hrs.
Sterility:	Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: negative
Biosafety Level:	1
Safety precautions:	If the cryovial is planned to be stored in liquid nitrogen and to be thawed in the future, special safety precautions should be followed: Protective gloves and clothing should be used and a facemask or safety goggles must be worn when transferring frozen samples into or removing from the liquid nitrogen tank. The removal of a cryovial from liquid nitrogen may result in the explosion of the frozen vial creating flying fragments. Caputo, J.L. Biosafety procedures in cell culture. J. Tissue Cult. Methods 11:223-227, 1988. ATCC Quality Control Methods for Cell Lines, 2nd edition, 1992.
Special Features of the Cell Line	
Viruses:	Tested negative for ectromelia virus (mouse pox). SMRV: Negative, as confirmed by Real-Time PCR

Authentication :	The mouse origin was verified by Real-Time-PCR.

Certificate of Analysis:	The Certificate of Analysis for each batch can be requested by e-mail at service@clsgmbh.de.

Recommendations for handling of cells growing in suspension following delivery	
Cryopreserved cells	The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial(s) must be stored below -150°C after arrival. If immediate culturing is intended, please follow these instructions: Quickly thaw by rapid agitation in a 37°C water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath. Note: A small ice clump should still remain and the vial should still be cold. From now on, all operations should be carried out under aseptic conditions. Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium (room temperature). Resuspend the cells carefully. Centrifuge at 300xg for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later. Resuspend the cells carefully in 10ml fresh cell culture medium and transfer them into one T25 cell culture flask. All further steps are described in the Subculture section.
Proliferating Cultures	The cell culture flask, 1xT25, comes filled with cell culture medium. Incubate at 37°C for a minimum of 24 hrs. Count the cells, spin down the cell suspension at 300x g for 3 minutes to collect the cells. Resuspend the cells in an appropriate amount of fresh cell culture medium and transfer to new cell culture flasks. Incubate at 37°C for a minimum of 24 hrs.

Warranty:	CLS warrants for a high cell viability and culture performance only if the product(s) is (are) stored and cultured according to the information described above. Using cell culture media and supplements other than the ones recommended in this product information may result in satisfactory proliferation and viabilities. CLS, however, does not warrant for cell recovery, proliferation and function if differing formulations are employed.
Disclaimer:	The customer shall not be entitled to employ this product for purposes other than research. Commercial utilization shall not be permitted; in particular, the cell line, its components or materials made therefrom shall not be sold or transferred to any third party. In addition, the term ‘Commercial use’ shall mean any activity by a party for consideration and may include, but is not limited to, use of the product or its components in manufacturing, for providing services, e.g. fee for service testing, in quality control or assurance processes within the manufacturing of products for sale, for therapeutic, diagnostic or prophylactic purposes, or for resale.