Designation: **P3/NS1/1-Ag4.1**

CLS order number: Cryovial: 400108
Vital: 440108

Origin and General Characteristics	
Synonym(s):	P3/NS1/1-AG4.1; NS1; NS-1
Organism:	Mus musculus (mouse)
Strain:	Balb/c
Morphology:	Myeloma; clone of P3X63Ag8
Growth Properties:	Suspension
Description:	Resistant to 8-azaguanine, no proliferation in HAT-selection medium.
Culture Conditions and Handling	
Culture Medium:	RPMI 1640 medium supplemented with 2mM L-glutamine, 1mM sodium pyruvate, sodium bicarbonate, and 10% fetal bovine serum (MG-70, CLS order number 820700).
Subculturing:	Maintain culture between 3-8 x10^5 cells/ml; Incubate at 5% CO_2, 37°C
Freeze Medium:	CM-5 (CLS order number: 800525, 25ml, 800550, 50ml)
Sterility:	Mycoplasma specific PCR: negative; Bacteria specific PCR: negative
Biosafety Level:	1
Special Features of the Cell Line	
Products:	The cells synthesise kappa-light chain of Immunoglobulin G (IgG1) but do not secrete.

References:

Kohler G, et al. Fusion between immunoglobulin-secreting and nonsecreting myeloma cell lines. Eur. J. Immunol. 6: 292-295, 1976.

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Recommendations for handling of adherent cell cultures following delivery

Cryopreserved cells

If immediate culturing is not intended, the cryovial(s) must be stored in liquid nitrogen (-196°C) or at least at -80°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 5ml fresh cell culture medium and transfer them into one T25 cell culture flasks.

All further steps are described in the Subculture section.

Proliferating Cultures

Collect the suspension cells by centrifugation at 300xg for 3 min and remove the supernatant carefully. Suspend the cell pellet in 5ml cell culture medium and determine the cell concentration. Distribute the cells at a starting concentration of $3x10^5$cells/ml. Incubate at 37°C for 24 hrs. As soon as a high cell viability is observed, the dilution factor may be increased.

Safety precautions for frozen cell lines

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 storing and/or thawing the cryovial.
- The removal of a cryovial from liquid nitrogen can result in the explosion of the cryovial creating flying fragments.

References: 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.