

**P3X63Ag8.653 Cells | 400118****General information**

**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.

**Organism** Mouse

**Tissue** Hematopoietic

**Disease** Myeloma

**Synonyms** P3-x63-Ag8.653, P3-x63-Ag8-653, P3-x63-Ag8 653, P3-x63-Ag 8.653, P3-x63Ag8.653, P3-x63.Ag8.653, P3/x63/Ag8.653, P3x63 Ag8.653, P3x63 AG8-653, P3x63-Ag8.653, P3x63-Ag8.653, P3x63 AG 8.653, P3x63Ag8653, P3-x63-Ag8-6-5-3, P3x63Ag8-6-5-3, P3.times.63 Ag8.653, P3.653, x63-Ag 8.6.5.3, x63-AG 8.653, x63-Ag8-653, x63-Ag8.653, x63.Ag8.653, x63Ag8-653, x63Ag8.653, x63AG8.653, P3-653, GM03570, GM3570, GM03570E, NS653

**Characteristics**

**Breed/Subspecies** BALB/c

**Gender** Female

**Morphology** Round cells

**Growth properties** Adherent/Suspension

**Regulatory Data**

**Citation** P3x63Ag8.653 (Cytion catalog number 400118)

**Biosafety level** 1

**NCBI\_TaxID** 10090

**CellosaurusAccession** CVCL\_4032

**Biomolecular Data**

**Viruses** Tested negative for ectromelia virus (mouse pox).

**P3X63Ag8.653 Cells | 400118****Handling****Culture Medium**RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO<sub>3</sub> (Cytion article number 820700a)**Supplements**

Supplement the medium with 10% FBS

**Dissociation Reagent**

Accutase

**Subculturing**

Gather the suspension cells in a 15 ml tube and gently wash the adherent cells with PBS lacking calcium and magnesium (use 3-5 ml for T25 flasks and 5-10 ml for T75 flasks). Apply Accutase (1-2 ml for T25 flasks, 2.5 ml for T75 flasks) ensuring full coverage of the cell layer. Allow the cells to incubate at room temperature for 10 minutes. Following incubation, combine and centrifuge both the suspension and adherent cells. After centrifugation, carefully resuspend the cell pellet and transfer the cell suspension into new flasks containing fresh medium.

**Seeding density**Start new cultures at  $4 \times 10^5$  cells/ml. The cell density should not exceed  $2 \times 10^6$  cells/ml.**Fluid renewal**

Every 3 to 4 days. Collect floating cells, centrifuge and add to the flask together with fresh medium.

**Post-Thaw Recovery**After thawing, plate the cells at  $5 \times 10^4$  cells/cm<sup>2</sup> and allow the cells to recover from the freezing process and to adhere for at least 48 hours.**Freeze medium**

As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

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### Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below  $-150^{\circ}\text{C}$  to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a  $37^{\circ}\text{C}$  water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at  $300 \times g$  for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

### Incubation Atmosphere

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

## Quality Control & Molecular Analysis

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**Sterility**

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.