

**RBL-2H3 Cells | 305194****Renseignements généraux****Description**

The RBL-2H3 cell line has become a valuable tool for studying mast cell physiology. RBL-2H3 cells express rat mast cell protease II (RMCP-II) and the c-kit receptor tyrosine kinase, making them a potential model for mast cells. However, conflicting and sometimes misleading data about RBL-2H3 cells have been reported.

RBL-2H3 cells have been widely used to investigate various aspects of mast cell function, including degranulation, mast cell stabilizers, and the interaction of FcεRI receptors with the cytoskeleton. They express high-affinity IgE receptors and can be activated to secrete histamine and other mediators. Cultivating RBL-2H3 cells is relatively easy, and longer culturing times result in higher cell density.

Degranulation is a key feature of RBL-2H3 cells, similar to mast cells and basophils. When allergens crosslink their IgE-bound FcεRI receptors, RBL-2H3 cells release preformed and newly synthesized mediators, contributing to immune allergic responses. The degranulation of RBL-2H3 cells has provided insights into basophil degranulation as well. These cells can also undergo degranulation in response to non-immunological stimuli, and there are differences between MMC, RBL-2H3, and CTMC.

The role of calcium in RBL-2H3 cell degranulation is significant. The calcium ionophore A23187, which increases intracellular calcium levels, induces degranulation in RBL-2H3 cells, similar to mast cells and basophils. Some studies have described RBL-2H3 cells as a serotonin-releasing cell line.

**Organism** Rat**Tissue** Peripheral blood**Disease** Rat leukemia**Synonyms** RBL2H3, RBL 2H3, RBL.2H3**Caractéristiques****Breed/Subspecies** Wistar**Morphology** Fibroblast**Growth properties** Adherent**Données réglementaires****Citation** RBL-2H3 (Cytion catalog number 305194)**Biosafety level** 1

**RBL-2H3 Cells | 305194****NCBI\_TaxID** 10116**CellosaurusAccession** CVCL\_0591**Données biomoléculaires****Manipulation****Culture Medium** EMEM (MEM Eagle), w: 2 mM L-Glutamine, w: 2.2 g/L NaHCO<sub>3</sub>, w: EBSS (Cytion article number 820100a)**Supplements** Supplement the medium with 10% FBS and 1% NEAA**Dissociation Reagent** Accutase**Subculturing** Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.**Fluid renewal** 2 to 3 times per week**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.

## Contrôle de la qualité et analyse moléculaire

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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.