

**RF/6A Cells | 305150**

**Renseignements généraux**

**Description**

RF/6A is a rhesus macaque (*Macaca mulatta*) retinal choroidal endothelial cell line established from fetal choroid and retina tissue. The line is registered in Cellosaurus as CVCL\_4552 and grows as an adherent monolayer with epithelial-like morphology. RF/6A cells retain key endothelial characteristics including expression of Factor VIII (von Willebrand factor), fibronectin, and Weibel-Palade granules detectable by electron microscopy — the latter confirming their endothelial identity. The line was originally established for studies of retinal and choroidal vascularization and has been widely adopted as a primate endothelial model for ocular angiogenesis research.

RF/6A is applicable in ocular angiogenesis research, studies of retinal and choroidal vascularization, evaluation of anti-angiogenic agents (VEGF inhibitors, bevacizumab, ranibizumab), age-related macular degeneration (AMD) modeling, diabetic retinopathy biology, and assessment of vascular permeability in the ocular microenvironment. The non-human primate (NHP) origin makes RF/6A closer to human retinal vascular biology than rodent endothelial models, particularly for studies involving primate-specific VEGF isoform responses and ocular pharmacology. The line is commonly used in tube formation assays, migration assays, and VEGF-stimulation experiments.

RF/6A is maintained as an adherent culture in EMEM supplemented with 10% FBS and 1% NEAA at 37°C in a humidified 5% CO<sub>2</sub> atmosphere. Cells are subcultured with Accutase at 70–80% confluency to prevent contact inhibition and loss of endothelial phenotype. Split ratio 1:3 to 1:5, seeding density 1–2 × 10<sup>4</sup> cells/cm<sup>2</sup>. Medium renewed 2–3 times per week.

**Organism** Rhesus macaque

**Tissue** Choroid, retina

**Disease** Normal retinal choroidal endothelium (fetal; non-tumorigenic)

**Metastatic site** Not applicable (normal fetal retinal choroidal endothelial cell line)

**Applications** Ocular angiogenesis research; retinal and choroidal vascularization; anti-VEGF therapy evaluation (bevacizumab, ranibizumab); AMD and diabetic retinopathy modeling; tube formation assays; vascular permeability; NHP primate retinal endothelial model

**Caractéristiques**

**Age** Fetus

**Gender** Sex unspecified

**Ethnicity** Not applicable (non-human primate cell line; *Macaca mulatta*)

**Morphology** Epithelial-like

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**Cell type** Endothelial cells**Growth properties** Adherent**Données réglementaires****Citation** RF/6A (Cytion catalog number 305150)**Biosafety level** 1**NCBI\_TaxID** 9544**CellosaurusAccession** CVCL\_4552**GMO Status** No genetic modification; wildtype rhesus macaque fetal retinal choroidal endothelial cell line**Données biomoléculaires****Protein expression** Factor  $\alpha$ , Fibronectin**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**Doubling time** approx. 24 to 36 hours**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.**Split ratio** 1 to 5

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**Seeding density** 1 to  $2 \times 10^4$  cells/cm<sup>2</sup>

**Fluid renewal** 2 to 3 times per week

**Post-Thaw Recovery** After thawing, plate the cells at  $5 \times 10^4$  cells/cm<sup>2</sup> and allow at least 24 hours for adherence before the first medium change. Do not allow cultures to reach full confluency as contact inhibition may reduce endothelial phenotype.

**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.

### 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°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°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 x 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°C, 5% CO<sub>2</sub>, humidified atmosphere.

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### 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

### 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.