Product sheet

Cytion

RF/6A Cells | 305150

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_2 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 × 10^4 cells/cm². 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

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

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

Protein expression	Factor [illegible], Fibronectin

Culture Medium	EMEM (MEM Eagle), w: 2 mM L-Glutamine, w: 2.2 g/L NaHCO3, 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 × 10^4 cells/cm^2
Fluid renewal	2 to 3 times per week
Post-Thaw Recovery	After thawing, plate the cells at 5 × 10^4 cells/cm^2 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_2, 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 °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 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

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.