**Product sheet**

# RF/6A Cells | 305150

## General information

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| **Description** | RF/6A is a rhesus macaque (Macaca mulatta) retinal choroidal endothelial cell line established from fetal choroid and retina tissue. The line is 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.<br><br>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. |
| **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 |

## Characteristics

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| **Age** | Fetus |
| **Gender** | Sex unspecified |
| **Ethnicity** | Not applicable (non-human primate cell line; Macaca mulatta) |
| **Morphology** | Epithelial-like |
| **Cell type** | Endothelial cells |
| **Growth properties** | Adherent |

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## Regulatory Data

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| **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 |

## Biomolecular Data

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| **Protein expression** | Factor VIII, Fibronectin |

## Handling

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| **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 |
| **Seeding density** | 1 to 2 × $10^4$ cells/$cm^2$ |
| **Fluid renewal** | 2 to 3 times per week |

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| **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.<br>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.<br>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.<br>4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.<br>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.<br>6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.<br>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.<br>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. |
| **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. |

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| **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. |

## Quality Control & Molecular Analysis

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| **Sterility** | Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.<br><br>To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections. |