



Human Retinal Microvascular Pericytes Expressing GFP-AEQ in Mitochondria

ORDER INFORMATION

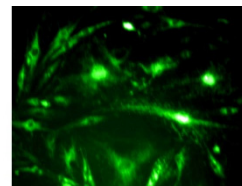
Name of Cells: Human Retinal Microvascular Pericytes Expressing GFP-AEQ in Mitochondria
Catalogue Number: cAP-0025GFP-AEQ
Product Format: Frozen Vial
Cell Number: > 3 x 10⁵/vial

General Information

HRMVPCs (cAP-0025) are initiated by elutriation from dissociated normal human retinal tissue. Puromycin resistant Mitochondria-GFP-HUVECs (cAP-0025GFP-AEQ) were selected and shipped in frozen vials (the cells are provided @ passage 3). Pericyte Growth Medium (cAP-09, containing FBS and growth factor supplements) is recommended for cell culture and these cells have a minimum of population doubling capacity > 10 when cultured following the detailed protocol described below.

Characterization of the cells

Cytoplasmic Alpha-Actinin Filaments > 80% positive by immunofluorescence
 Cytoplasmic Desmin Intermediate Filaments > 80% positive by immunofluorescence
 Cytoplasmic VWF / Factor VIII < 2% positive by immunofluorescence
 Cytoplasmic uptake of Di-I-Ac-LDL < 2% positive by immunofluorescence
 HRMPCs are negative for HIV-1, HBV, HCV, and mycoplasma.



Human Retinal MV Pericytes expressing GFP-AEQ in Mitochondria

Product Use: cAP-0025GFP-AEQ cells are for **Research Use Only.**

Shipping status: Proliferating cells in T25 flask or in a frozen vial.

Handling of Arriving Cells

When you receive the cells in a frozen vial, you can transfer the vial of cells into a -80°C freezer for short period storage or a liquid nitrogen tank for long term storage. Thaw the cells in a 37°C water bath, and then transfer the cells in a T25 flask pre-coated with Quick coating solution (cAP-01) as described in details in Subculture Protocol.

1. Subculture Protocol:

- Pre-coating of T25 flasks: Add 2ml of Quick Coating Solution (cAP-01) into one T25 flask and make sure whole surface of the flask is covered with the coating solution. Five minutes later, dispose excessive Quick Coating Solution by aspiration and the flask is ready to be used (no need for overnight incubation when using Quick Coating Solution). Other extracellular matrix can be used including gelatin, collagen, and fibronectin and you are advised to test the conditions for using those materials in advance.
- Rinse the cells in T25 flask with 5ml HBSS (**Room Temperature, RT**) twice.
- Add 2ml of Trypsin/EDTA (**RT**) (cAP-23) into one T25 flask (make sure the whole surface of the T25 flask is covered with Trypsin/EDTA), and gently dispose the excessive Trypsin/EDTA solution **within 20 seconds** with aspiration.
- Leave the T25 flask with the cells at **RT** for 1 minute (the cells usually will detach from the surface within 1-2 minutes). You can monitor the cells under microscope and when most of cells become rounded up, hit the flask against the bench surface, and the cells will move on the surface of the flask when monitoring under microscope.
- Add 5ml Trypsin Neutralization Buffer and spin the cells down with 800g for 5 minutes.
- Re-suspend the cell pellet with 10 ml of PGM full medium and the cell suspension is transferred directly into 2 pre-coated T25 flasks (5ml each, and the cells are sub-cultured at 1:2 ratio)
- Change medium every 2-3 days and cells usually become confluent within 7 days (when split at a 1:2 ratio).

Related products

Quick Coating Solution	cAP-01	240ml	Angio-Proteomie
Pericyte Growth Medium	cAP-09	500ml	Angio-Proteomie
Pericyte Growth Medium (super rich formulation)	cAP-09B	500ml	Angio-Proteomie
Pericyte Basal Medium	cAP-09C	500ml	Angio-Proteomie
HBSS w/o Ca ²⁺ , Mg ²⁺	cAP-11	100ml	Angio-Proteomie
Cell Freezing Solution (FBS)	cAP-22	50ml	Angio-Proteomie
Cell Freezing Solution (Non-FBS)	cAP-22B	50ml	Angio-Proteomie
Trypsin/EDTA Solution	cAP-23	100ml	Angio-Proteomie
Trypsin Neutralization Solution	cAP-28	100ml	Angio-Proteomie
ITS (100x)	cAP-26	10ml	Angio-Proteomie
L-Glutamine-MAXIMUM (100x)	cAP-27	100ml	Angio-Proteomie
Human Plasma Fibronectin Solution	cAP-42	1mg/ml	Angio-Proteomie



Activation Assay Using cAP-0025GFP-AEQ (Human Retinal Microvascular Pericytes expressing mitochondrial-targeted GFP- GPCR Aequorin)

I. Product Overview

cAP-0025GFP-AEQ consists of Human Retinal Microvascular Pericytes stably expressing mitochondrial-targeted GFP-Aequorin, enabling highly sensitive, real-time monitoring of GPCR-induced mitochondrial calcium signaling.

II. Principle of the Assay

Activation of GPCRs on pericytes triggers intracellular calcium mobilization, driving calcium uptake into mitochondria, which is detected by mitochondrial-targeted GFP-Aequorin. The luminescent signal generated corresponds directly to mitochondrial calcium concentration and GPCR activation.

III. GPCRs Commonly Expressed in Retinal Pericytes

- Platelet-derived growth factor receptor β (PDGFR β)
- Angiotensin receptors (AT₁R, AT₂R)
- Endothelin receptors (ETA, ETB)
- Protease-activated receptors (PAR-1, PAR-2)
- Adenosine receptors (A_{2A}, A_{2B})
- Sphingosine-1-phosphate receptors (S1P₁, S1P₂)
- Vasopressin receptor (V1a)

IV. Materials Needed

- cAP-0025GFP-AEQ cells
- Coelenterazine (5 μ M)
- GPCR agonists and antagonists
- HBSS buffer (calcium-containing)
- Clear-bottom, white-wall 96-well luminescent assay plates
- Plate reader/luminometer capable of luminescence detection
- Pericyte Growth Medium (PGM, cAP-09)

V. Protocol Steps

1. Cell Preparation:

- Culture cAP-0025GFP-AEQ cells using Pericyte Growth Medium (PGM, cAP-09).
- Seed 2×10^4 cells per well into luminescent assay plates; incubate overnight until $\sim 80\%$ confluency.

2. GFP-Aequorin Activation (Coelenterazine Loading):

- Replace medium with PGM containing 5 μ M Coelenterazine.
- Incubate in the dark at 37°C for 2 hours.

3. Cell Washing:

- Wash cells gently 2 times with calcium-containing HBSS buffer.
- Equilibrate cells in HBSS buffer for 15-30 minutes at 37°C.



4. GPCR Stimulation and Luminescence Measurement:

- Transfer plates promptly to a luminometer.
- Inject GPCR agonists (final volume 100 μ L per well).
- Record luminescence every second for 60–120 seconds.

Suggested GPCR agonist concentrations:

- Angiotensin II: 0.1–1000 nM
- Endothelin-1: 0.1–100 nM
- PDGF-BB: 0.1–50 ng/mL
- S1P: 10 nM–10 μ M

VI. Controls

- Positive control: ATP (10 μ M)
- Negative control: Vehicle-only (HBSS)

VII. Data Analysis

- Express data as Relative Luminescence Units (RLU).
- Normalize luminescence readings to baseline.
- Generate dose-response curves; calculate EC_{50} or IC_{50} .

VIII. Applications

- Pharmacological profiling of GPCR agonists and antagonists
- GPCR signaling pathway investigation
- Retinal pericyte physiology and vascular research

Utilize cAP-0025GFP-AEQ for precise and physiologically relevant assessments of mitochondrial calcium signaling mediated by GPCRs in human retinal pericytes.

Caution: Handling human tissue derived products is potentially bio-hazardous. Although each cell strain is tested negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate; therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.