ANGIO-PROTEOMIE

DATA SHEET

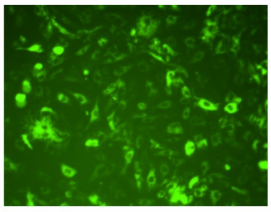
Human Brain Microvascular Endothelial Cells Expressing GFP-AEQ in Mitochondria

ORDER INFORMATION

Name of Cells: Human Brain Microvascular Endothelial Cells Expressing GFP-AEQ in Mitochondria
Catalogue Number: **cAP-0002GFP-AEQ**
Product Format: Frozen Vial
Cell Number: Frozen Vial (> 5 x 10^5/vial)

General Information
HBMVECs (**cAP-0002**) cells were isolated from normal human brain tissue. Puromycin resistant Mitochondria-AEQ-GPF-HBMVECs (cAP-0002GFP-AEQ) were selected and shipped in frozen vials (the cells are provided @ passage 3).The cells are shipped in frozen vials (the cells are provided @ passage 2). Endothelial Growth Medium (EGM, contains 10% serum and growth supplements, cAP-02) is recommended for cell culture and these cells have a minimum average population doubling levels > **15** when cultured following the detailed protocol described below).

Characterization of the Cells
Cytoplasmic VWF / Factor VIII: **>95% positive by immunofluorescence**
Cytoplasmic uptake of Di-I-Ac-LDL: **>95% positive by immunofluorescence**
Cytoplasmic PECAM1: **>95% positive by immunofluorescence**
HBMVECs are negative for HIV-1, HBV, HCV, and mycoplasma.



Human Brain MVEC expressing GFP-AEQ in Mitochondria

Shipping: Proliferating culture in T25 flask or Proliferating cells in T25 or a Frozen Vial.
Product Use: cAP-0002GFP-AEQ cells are for research use only.

Handling of Arriving Cells
When you receive the cells in a frozen vial, you can transfer the vial of cells into a -80ºC 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 into a T25 flask pre-coated with Quick coating solution (cAP-01) as described in details in Subculture Protocol.

Subculture Protocol
A) 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.
Note: For certain endothelial cells, fibronectin (cAP-42) coated culture wares are essential (check cAP-42 for detailed protocols).
B) Rinse the cells in T25 flask with 5ml HBSS (**Room Temperature, RT**) twice.
C) 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.
D) 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.
E) Add 5ml Trypsin Neutralization Buffer and spin the cells down with 800g for 5 minutes.
F) Re-suspend the cell pellet with 10 - 15ml of EGM full medium and the cell suspension is transferred directly into 2 or 3 pre-coated T25 flasks (5ml each, and the cells are sub-cultured at 1:2 to 1: 3 ratios)
G) Change medium every 2-3 days and cells usually become confluent within 7 days (when split at a 1:3 ratio).
H) If you need prepare quiescent cells, when cells are almost confluent, replace EGM full medium with Endothelial Basal Medium (EBM, cAP-03) containing 0.5% FBS about 8-12 hours before your experiments.

Related products

Quick Coating Solution	cAP-01	240ml	Angio-Proteomie
Endothelial Growth Medium	cAP-02	500ml	Angio-Proteomie
Endothelial Basal Medium	cAP-03	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

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GPCR Activation Assay Protocol
Cell Line: cAP-0002GFP-AEQ (Human Brain Microvascular Endothelial Cells Expressing GFP-Aequorin in Mitochondria)
Culture Medium: Endothelial Cells Growth Medium (cAP-02)

Product Overview

The cAP-0002GFP-AEQ cells are human brain microvascular endothelial cells (MVECs) genetically engineered to express mitochondria-targeted GFP-Aequorin (GFP-AEQ), enabling real-time monitoring of intracellular calcium signals upon GPCR activation. These cells serve as a robust cellular model for studying GPCR signaling pathways relevant to the human blood-brain barrier (BBB).

Principle of the Assay

This assay utilizes mitochondria-targeted GFP-Aequorin, a calcium-sensitive luminescent protein, to measure intracellular calcium flux in response to GPCR activation. When GPCRs are activated by specific ligands, calcium is released from intracellular stores or enters from the extracellular space, leading to a luminescent emission detectable by a microplate reader. The intensity of the emitted light correlates directly with GPCR activation and subsequent intracellular calcium signaling events.

GPCRs Commonly Expressed in Brain MVECs

Brain MVECs commonly express various GPCR families including:

- **Purinergic receptors (P2Y)**
- **Histamine receptors (H1, H2)**
- **Bradykinin receptors (B2)**
- **Adrenergic receptors (α2, β1, β2)**
- **Serotonin receptors (5-HT)**
- **Protease-activated receptors (PAR-1, PAR-2)**
- **Sphingosine-1-phosphate receptors (S1P1, S1P3)**
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These receptors regulate vital physiological processes at the blood-brain barrier, such as vascular tone, permeability, inflammation, and endothelial cell integrity.

Materials

- **cAP-0002GFP-AEQ cells**
- **Endothelial Cells Growth Medium (cAP-02)**
- **HBSS (with calcium)**
- **GPCR-specific agonists**
- **Positive control agonists: ATP (100 µM), Histamine (100 µM), Thrombin (1 U/ml)**
- **Negative control: HBSS alone**
- **Luminescence microplate reader**
- **White-wall clear-bottom 96-well assay plates**

Protocol

Step 1: Cell Preparation

1. **Plate cAP-0002GFP-AEQ cells at 20,000–30,000 cells/well in white-wall clear-bottom 96-well plates.**

2. **Culture in cAP-02 medium at 37°C, 5% CO_2 until ~90% confluent (24–48 hours).**

Step 2: Assay Buffer Preparation

- **Prepare HBSS supplemented with 1.8 mM $CaCl_2$. Warm buffer to 37°C.**

Step 3: Cell Equilibration

1. **Wash cells once with HBSS.**
2. **Add 100 µl HBSS per well, incubate at 37°C for 30–45 min.**

Step 4: Luminescence Measurement (GPCR Stimulation)

1. **Prepare 2X ligand solutions.**
2. **Set luminescence reader to kinetic mode (1 sec intervals, 60–120 sec total).**
3. **Inject 100 µl ligand solution rapidly per well (final volume: 200 µl).**
4. **Immediately measure luminescence.**

Step 5: Data Analysis

- **Export luminescence data.**
- **Normalize responses to negative control.**
- **Plot dose-response curves, calculate EC_{50} values.**
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Experimental Plate Layout Example

Condition	Replicates	Description	Final Concentration
Negative Control	3–4	Buffer alone	–
Positive Control	3–4	ATP, Histamine, Thrombin	100 µM (ATP/Histamine), 1 U/ml (Thrombin)
GPCR Agonists	3–4 per dose	GPCR-specific ligands	10 nM–10 µM

Notes

- **Rapid ligand addition and immediate measurement are essential.**
- **Conduct the assay consistently at 37°C.**
- **GPCR-specific antagonists can validate receptor-specific responses.**