

## RFP-Expressing Bovine Aortic Endothelial Cells

### ORDER INFORMATION

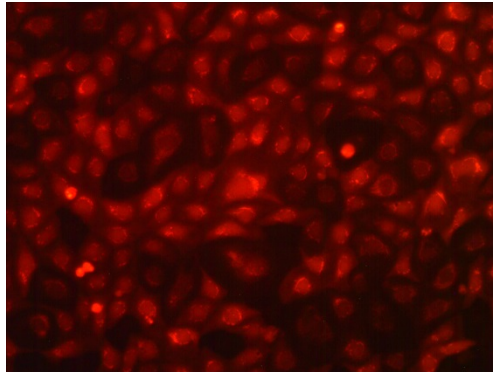
**Name of Cells:** RFP Expressing Bovine Aortic Endothelial Cells (**RFP-bAECs**)  
**Catalogue Number:** **cAP-b0001RFP**  
**Product Format:** Proliferating culture or Frozen vials  
**Cell Number:** > 90% confluent in T25 flask or >5 x 10<sup>5</sup>cells/vial

### General Information:

**RFP-bAECs (cAP-b0001RFP)** are selected from bAECs (cAP-b0001) transfected using lenti-virus expressing RFP using Puromycin. The cells are shipped in proliferating culture with >90 confluence (the cells are provided @ passage 2) or in frozen vial (with > 5 x 10<sup>5</sup>cells/vial). Endo-Growth Medium (cAP-02) is recommended for the expansion of bAECs and these cells can be propagated to sixth passage and beyond without losing their morphologic and phenotypic characteristics when cultured following the detailed protocol described below).

### Characterization of the cells

PECAM1: >**95% positive by immunofluorescence**  
VE-Cadherin: >**95% positive by immunofluorescence**  
negative for mycoplasma.



**Product Use:** RFP-bAECs are for research use only.

**Shipping:** Proliferating culture in T25 flask or Frozen vials.

### Handling of Arriving Cells

**Proliferating culture)**

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When you receive the cells, leave the flask in 37°C CO<sub>2</sub> incubator for 1 hour first, and then replace the transport medium with Endo-growth medium. Let the cells to grow for 24 hour before subculture if the cells are not completely confluent.

#### Frozen vials

When you receive the frozen vials, you can keep the frozen vials in a -80°C Freezer for short term storage or in a liquid nitrogen tank for long term storage. Frozen vials should be thawed in 37°C water bath immediately before plating the cells in to 10ml of Endo-Growth medium in a T25 flask, and the cells should be incubated in a 37°C CO<sub>2</sub> incubator for overnight. The medium should be changed on the next day.

### 1. Subculture Protocol:

- A) Rinse the cells in T25 flask with 5ml DPBS (**Room Temperature, RT**) twice.
- B) Add 2ml of Trypsin/EDTA (**RT**) (Invitrogen Catalogue number: 25300-062) into T25 flask (make sure the whole surface of the T25 flask is covered with Trypsin/EDTA), and gently dispose the Trypsin/EDTA solution **within 10 seconds** with aspiration.
- C) Leave the T25 flask with the cells at **RT** for 1-2 minute (the cells will normally come off the surface within 1 minute, monitor the cell under microscopy).
- D) Suspend the cells with 20ml of Endo-Growth Medium and then split cell suspension into 2 T25 flasks (10ml each, and the cells are subcultured at 1:2 ratio)

### 2. Cell culture protocol (proliferating):

- A) Endo-growth medium should be changed every other day.
- B) The cells normally become confluent within 5-6 days (when split at 1:2 ratio).

Although primary cells are tested pathogen-free, investigators should handle these cells with caution and treat all animal cells as potential pathogens, since no test procedure can completely guarantee the absence of infectious agents.

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