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GFP Expressing Human Retinal Microvascular Pericytes

ORDER INFORMATION

Name of Cells: GFP Expressing Human Retinal Microvascular Pericytes Cells **(GFP-HRMPCs)**
Catalogue Number: **cAP-0025GFP**
Product Format: Proliferating culture
Cell Number: > 90% confluent (> 3 x 10^5 cells) in T25 flask

General Information

HRMPCs (**cAP-0025**) are initiated by elutriation from dissociated normal human retinal tissue and transfected with GFP-Lentiviral particles at passage two. Puromycin resistant GPF-HRMPCs (cAP-0025GFP) were selected and shipped in proliferating culture with >90 confluence (the cells are provided @ passage 3-5). DMEM medium (contains 10% serum and growth supplements) is recommended for cell culture and these cells have an average 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.

Product Use

GFP-HRMPCs are for research use only.

Shipping status

Proliferating cells in T25 flask.

Handling of Arriving Cells

When you receive the cells, leave the flask in 37°C CO2 incubator for 1 hour first, and then replace the transport medium with fresh DMEM medium (contains 10% serum and growth supplements). Let the cells grow for 24 hours before subculture.

1. Subculture Protocol:

A) Coating T25 flasks: Add 2ml 0.1% 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 Quick Coating Solution by aspiration and the flask is

ready to be used (no need for overnight incubation when coated with Quick Coating Solution).

B) Rinse the cells in T25 flask with 5ml PBS (**Room Temperature, RT**) twice.

C) 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 Trpsin/EDTA solution **within 10 seconds** with aspiration.

D) Leave the T25 flask with the cells at **RT** for 1 minute (the cells will normally come off the surface within 1 minute).

E) Suspend the cells with 20ml of DMEM medium (contains 10% serum and growth supplements) and the cell suspension is transferred directly into 4 x pre-coated T25 flasks (5ml each, and the cells are subcultured at 1:4 ratio)

(Note: No need spin the cells during the subculture process).

2. Cell culture protocol (proliferating):

A) Culture medium DMEM medium (contains 10% serum and growth supplements) is changed every 2 days.

B) The cells normally become confluent within 7 days (when split at 1:4).

3. Preparation of quiescent cells:

A) DMEM-basal medium containing 0.5% FBS is used to induce quiescent pericytes (after 18-24hours).

Other products needed:

Items	Company	Cat #
Quick Coating Solution	Angio-Proteomie	cAP-01
PBS	Invitrogen	10010
Trypsin/EDTA	Invitrogen	25300-062