ANGIO-PROTEOMIE

DATA SHEET

RFP-Expressing Conditionally Immortalized Human Glomerular Podocytes (SV40, Tet-On)

ORDER INFORMATION

Name of Products: RFP-Expressing Conditionally Immortalized Human Glomerular Podocytes (RFP-CI-HGlPod Cells)
Catalogue Number: **cAP-0064RFP**
Product Format: Frozen Vial
Cell Number: > 5 x 10^5/vial

General Information
Human Primary Glomerular Podocytes were initially isolated and expanded from healthy kidney biopsy. Conditionally immortalized Human Glomerular Podocytes (**CI-HGlPod Cells**) are selected by puromycin after primary human podocytes are infected with the lentiviral particles expressing SV40 under the control of CMV promoter with the Ten-on system. RFP-CI-HGlPod Cells were selected by Zeocin after CI-HGlPod Cells are infected with lentiviral particles expressing RFP. Podocyte Growth Medium (cAP-47) is recommended for cell culture and these cells have an average minimum population doubling levels > 50, when cultured following the detailed protocol described below).

Characterization of the cells

1. The cells are confirmed positive for CD2AP and Neprin
2. CI-HGlPod Cells are tested negative for HIV-1, HBV, HCV, and mycoplasma.

Product Use: RFP-CI-HGlPod Cells are for Research Use Only.

Shipping: Frozen vials in dry ice package.

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, in 9 ml Human Podocytes Growth Medium (cAP-47). (Make sure to add 2.0ug/ml of **Doxycycline,** cAP-45).

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.
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 Human Tendon Cell Medium (cAP-47) and the cell suspension is transferred directly into 2 or 3 pre-coated T25 flasks (10ml each, and the cells are sub-cultured at 1:2 to 1: 3 ratios). (Make sure to add 0.025ug/ml of **Doxycycline**).
G) Change medium every 2-3 days and cells usually become confluent within 7 days (when split at a 1:2 or 1:3 ratio).

Notes
1. The cells are proliferative better if the cells are maintained at 33-35°C rather than 37C.
2. To induce cellular quiescence, withdrawing DOX and maintain the cells at 37°C for 3-5 days.

Related products

Quick Coating Solution	cAP-01	240ml	Angio-Proteomie
Podocytes Growth Medium	cAP-47	500ml	Angio-Proteomie
Podocytes Basal Medium	cAP-47B	500ml	Angio-Proteomie
HBSS w/o Ca^{2+} , Mg^{2+}	cAP-11	100ml	Angio-Proteomie
Trypsin/EDTA Solution	cAP-23	100ml	Angio-Proteomie
Trypsin Neutralization Solution	cAP-28	100ml	Angio-Proteomie
Doxycycline Solution (1000 x)	cAP-45	10ml	Angio-proteomie

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.

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