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DATA SHEET

Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture - T225 Plated Cells

Catalog number: 46015-02-T225

Description: Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture T225 Plated Cells. Also available in T25, T75, and T150 tissue culture flask with plated cells, shipped at room temperature or in Frozen Vial, shipped in dry ice. The Clonal Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture was derived from Dog Lung Alveolar. They were maintained in Celprogen's Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture Complete Growth Medium and sub-cultured every 24 to 48 hours on Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture Extra-cellular Matrix.

Source: Dog Lung Alveolar

Mycoplasma test: Negative-PCR and mycoplasma agar methods

Sterility: Negative for bacteria, yeast, and mold

Storage Conditions: Liquid nitrogen vapor phase for frozen Ampule of Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture .

For plated cells in tissue culture flask, upon receipt of the cells wipe the flask with 70% ethanol and transfer to sterile tissue culture hood. In the tissue culture hood remove the media from the cells and wash the cells with 1X PBS sterile solution, for 2-3 minutes, remove the 1X PBS solution and then Trypsinize. After Trypsinization of the Cells neutralize the Trypsin with equal volume of Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture Complete Growth Media with Serum and collect the Cell suspension in sterile conical centrifuge tube in the tissue culture hood. Centrifuge the cell suspension at 100g for 7 minutes in centrifuge. Plate cells $5x10^5$ cells per pre-coated flasks with Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture Extra-cellular Matrix for Expansion in Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture Complete Growth Medium.

Positive Markers: Type 1 pnuemocytes: Caveolin 1 & 2 Aquaporin E & T1alpha

Morphology & Proliferation: Mixed population of cells with approximately 95% attached cells and the other 5.0% in suspension, need to change cell culture media every day after 48 hours of initial cell culture or when the media starts changing color to slight yellow from pink. Fast growing cell culture, change media with Celprogen's Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture Complete Growth Medium with the appropriate Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture Extra-cellular Matrix. Temperature $37^{O}C$ in 5% CO_2 humidified incubator.

Sub-culturing:

1. Remove flask and wipe with 70% ethanol. Then transfer to the tissue culture hood. Refer to protocols, flow diagrams and videos for more detail.
2. Transfer the supernatant contents to a sterile centrifuge tubes, this will contain detached cells.Centrifuge at 100g for 7 minutes to obtain cell pellet. Plate cells in a T225 flask pre-coated with ECM.
3. Add pre-warmed Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture Growth Media to flask that has attached cells and incubate for two hours in the incubator. After 2 hours wash the cells with 1X PBS, remove
100% 1X PBS solution.

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4. Trypsinize the attached cells with 3ml 1X Trypsin EDTA for 2-3 minutes. At the end of Trypsinization add equal volume of complete growth media with serum. Centrifuge the cells at100g for 7 minutes to obtain cell pellet.

5. Add the 500ul of cells to T225 flask pre-coated with Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture Extra-cellular Matrix with 7ml of Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture Complete Growth Medium. Depending on the size of the pellet you may utilize a new flask or you may combine the cells to the flask that had the supernatant cells.

6. Incubate the cells in the T225 flask at 37^0C in a 5% CO_2 humidified incubator. Perform 100% Media Change every 24 to 48 hours.

7. Medium renewal every other day or 2-3 days, sub-culturing ratio: 1:2 or 1:3 depending on the cell density.

8. Refer to protocols, flow diagrams and videos for more detail. http://celprogen.com/tech.htm

Freezing Medium: Available for purchase Cat# M46015-02FM

Trypsin: Available for purchase Cat# T1509-014

IX PBS: Available for Purchase Cat# P1408-013

Storage temperature: Liquid nitrogen vapor phase

Product Orders: Before submitting an order you will be asked to read and accept the terms and conditions of Celprogen's Material Transfer Agreement (MTA).

Permits/Forms: In addition to the MTA mentioned above, other CELPROGEN and/or regulatory permits may be required for the transfer of this CELPROGEN material. Anyone purchasing CELPROGEN material is ultimately responsible for obtaining the permits.

Biosafety Level: 1

Notices & Disclaimers: ***CELPROGEN products are intended for laboratory research purposes only*. They are not intended for use in Humans.** The product, Canis Familaris (Dog) Alveolar Type 1 Primary Cell Culture , is established and manufactured by CELPROGEN Inc, and is for Research Use Only. This product is not for re-sale or may not be transferred to a third party prior to written request and approval by CELPROGEN Inc.