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## **DATA SHEET**

**Product name:** Canis Familaris (Dog) Alveolar Primary Cell Culture - Frozen Vial

Catalog number: 46014-01

**Description:** Frozen Ampule  $(1.2 \times 10^6 \text{ Cells})$  of  $1 \times 10^6 \text{ viable cells upon thawing, shipped}$ 

with dry-ice. Also available in T25, T75, T150, and T225 tissue culture flasks with plated cells, shipped at room temperature. They were maintained in Celprogen's Canis Familaris (Dog) Alveolar Primary Cell Culture Complete Growth Medium and sub-cultured every 24 to 48 hours on Canis Familaris

(Dog) Alveolar Primary Cell Culture Extra-cellular Matrix.

**Source:** Canis Familaris Alveolar Tissue

Storage Conditions: Liquid nitrogen vapor phase for frozen Ampule of Canis Familiaris (Dog)

Alveolar 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 of the cells and wash the cells with 1X PBS sterile solution, for 2-3 minutes, remove the PBS solution and then trypsinize. After trypsinization of the Cells neutralize the trypsin with equal volume of Canis Familaris (Dog) Alveolar 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<sup>5</sup> cells per pre-coated flasks with Canis Familaris (Dog) Alveolar Primary Cell Culture Extra-cellular Matrix for Expansion in Canis Familaris (Dog) Alveolar Primary Cell Culture

Cell Complete Growth Medium.

Mycoplasma test: Negative-PCR and mycoplasma agar methods

Sterility: Negative for bacteria, yeast, and mold

**Positive Markers:** ESA, CD18, Alkaline Phosphatase, SBA, SPB

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 for pink. Fast growing cell culture. Change media with Celprogen's Canis Familaris (Dog) Alveolar Primary Cell Culture (mixed with type 1 and type 2 cells) Complete Growth Medium with the appropriate Canis Familaris (Dog) Alveolar Primary Cell Culture (mixed with type 1 and type 2 cells) Extracellular Matrix. Temperature 37°C in 5% CO<sub>2</sub> humidified incubator. Population doubling is 120 or up to 12 passages when cultured at 60% to70% confluent on Celprogen Culture System.



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## **Sub-culturing:**

- 1. Thaw the vial with gentle agitation in a 37°C water bath or a dry 37°C shaking incubator. For water bath thawing keep the O-ring out of the water.
- 2. Remove the thawed vial and wipe with 70% ethanol. Then transfer to the tissue culture hood.
- 3. Transfer the vial contents to a sterile centrifuge tube, and gently add pre-warmed Canis Familaris (Dog) Alveolar Primary Cell Culture Growth Media to the centrifuge tube. Use additional Canis Familaris (Dog) Alveolar Primary Cell Culture Complete Media to rinse the vial and transfer the liquid to the centrifuge tube repeat this once more to ensure you have all the cells transferred to the 15ml centrifuge tube. Centrifuge the cells at 100g for 7 minutes. Remove the supernatant and resuspend the cell pellet in 500ul of Canis Familaris (Dog) Alveolar Primary Cell Culture Complete Growth Medium.
- **4.** Add the 500ul of cells to T75 flask pre-coated with Canis Familiaris (Dog) Alveolar Primary Cell Culture Extra-cellular Matrix with 15ml of Canis Familiaris (Dog) Alveolar Primary Cell Culture Complete Growth Medium.
- 5. Incubate the cells in the T75 flask in a 37°C in 5% CO<sub>2</sub> humidified incubator. Perform 100% Media Change every 24 to 48 hours.
- **6.** Medium renewal every other or 2-3 days, sub-culturing ratio: 1:3

**Freezing Medium:** Available for purchase Cat# M46014-01FM

**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.

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