



## GFP Expressing Human Dermal Fibroblasts - Neonatal

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**Catalog #:** GFP07

**Cell #:** >5x10<sup>5</sup> cells

**Storage:** Liquid Nitrogen until ready for culture.  
While Culturing keep in 37°C CO<sub>2</sub> incubator

**Product Format:** Cryopreserved

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### GENERAL INFORMATION

HNDFCs-Neo cells are isolated from normal neonatal foreskin tissue samples and transfected with GFP-Lentiviral particles at passage one. Puromycin resistant GFP-HNDFCs-Neo are selected and shipped in frozen vial (the cell is provided @passage 1). Fibroblast Growth medium (F001, full medium) is recommended for cell culture and these cells have an average minimum population doubling levels > 14 when cultured following the detailed protocol described below). HNDFCs-Neo are tested negative for HIV-1, HBV, HCV, and mycoplasma.

*Product is for Research use only*

Cells are shipped on dry ice

### HANDLING OF ARRIVING CELLS

When you receive the dry ice package with cells in frozen vials, transfer the frozen vials of cells into a -80C freezer for short period storage or a liquid nitrogen tank for long- term storage.

### PROTOCOL FOR THAWING THE CELLS AND SUBCULTURE

1. Pre-coating of T25 flasks- Add 2ml of AlphaBio Coat solution into a T25 flask to cover the whole surface of the flask, 5 mins later, dispose the excessive coating solution by aspiration and the flask is ready to be used.
2. Thaw the frozen cell vial in a 37C water bath first, and then transfer the cells into the pre-coated T25 flask with 10ml of cAP-44 medium, cells usually become confluent with 1-2 days and ready to be passaged.
3. To passage the cells, rinse the cells in a T25 flask with 5ml HBSS (RT) twice; then add 2ml Cell Detachment solution (RT) into one T25 flask; gently dispose the excessive detachment solution within 20 seconds by aspiration.
4. Leave the T25 flask with the cells at RT or 37C for 1 min (most cells usually will detach from the surface within 1-2 mins; or monitor the cells under a microscope until most of cells become rounded up, and then gently tap the flask against the bench surface, and the cells will move on the surface of the flask when monitoring under microscope
5. Leave the T25 flask with the cells at RT or 37C for 1 min (most cells usually will detach from the surface within 1-2 mins; or monitor the cells under a microscope until most of cells become rounded up, and then gently tap the flask against the bench surface, and the cells will move on the surface of the flask when monitoring under microscope.
6. Add 5ml Trypsin Neutralization Buffer and spin down the cells with 800g centrifugation for 5 mins.

### FOR RESEARCH USE ONLY

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7. Re-suspend the cell pellet with 10 or 15ml F001 medium and transfer 5 ml each into 2 or 3 pre-coated T25 flasks (for 1/2 to 1/3 subculture ratio).
8. Change medium every 2 or 3 days and the cells usually become confluent within 7 days (when split at a 1/3 ratio).
9. To prepare quiescent cells, when cells are nearly confluent, replace with Fibroblast Basal Medium (F002) containing 0.5% FBS for about 8-12 hrs before your experiments.

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