Product Name: **hTERT Cell Immortalization Kit** Cat. #: **hTERT**

Description:
It is well known that primary cells only undergo a pre-determined and finite number of cell divisions in culture. After limited population doublings (the number of which varies by species, cell type, and culture conditions), primary cells enter a state, so-called replicative senescence, where they can no longer divide. To surpass senescence, several methods exist for immortalizing mammalian cells in culture. The most recently discovered approach to cell immortalization is through the expression of Telomerase Reverse Transcriptase protein (TERT). It is particularly useful for cells that are most affected by telomere length, including many human cell types. This protein is usually silenced in most somatic cells. These cells can avoid replicative senescence by maintaining sufficient telomere lengths when hTERT is exogenously introduced. However, over-expression of hTERT in some cell types (especially in epithelial cells) fails to induce cell immortalization.

Size: 5 x 20 µl **Ship:** Dry ice **Store at** -80 °C (stable up to 6 months)

Quality Control: The packaged lentivector constructs are provided as frozen VSV-G pseudotyped viral particles. The titer of the lentivirus is measured by Clontech's Lenti-X qRT-PCR Titration Kit (Cat. # 631235). Each lot of Cell Immortalization kit is functionally tested in fibroblasts.

Safety Precaution: We highly recommends that protective gloves, a lab coat, and a full-face mask always are worn when handling frozen vials. It is important to note that some liquid nitrogen can leak into the vials when submersed in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in excessive pressure within the vial that can cause the vial to explode or expel the cap with dangerous force. This kit is **for research use only.** Not for use in diagnostic or therapeutic procedures.

Protocol

1. Plate the target cells in one well of 6-well plate at density of 1-2 x 105 cells/well.
2. The next day, take one vial of the concentrated recombinant lentivirus from -80 °C freezer and thaw it on ice.
3. Infect the target cells in a 6-well plate with 4-20 µl/well viral supernatant in the presence of 4 µl of Transduction Enhancer Reagent (101Bio, Cat.#: PV500), which is a polycation that neutralizes charge interactions to increase binding between the pseudoviral capsid and the cellular membrane.
4. The next day, aspirate medium containing viral supernatant and add the appropriate complete growth medium to the cells and incubate at 37 °C.
5. After 72 hours incubation, subculture the cells into 2 x 100 mm dishes and add the appropriate amount of puromycin for stable cell-line generation.
6. 10-15 days after selection, pick clones for expansion and screen for positive ones. Note: Since the virus-titer will decrease significantly, we recommend that adding 25% v/v virus protection medium (101Bio, Cat.#: PV500) into the thawed supernatant before frozen again for future use.

-- The end --