Product Name: **SV40 T Antigen Cell Immortalization Kit** Cat. #: **SV40T**

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, socalled replicative senescence, where they can no longer divide. To surpass senescence, several methods exist for immortalizing mammalian cells in culture. Simian virus 40 (SV40) T antigen has been shown to be **the simplest and most reliable** agent for the immortalization of many different cell types. Recent studies have also shown that SV40 T antigen can induce Telomerase activity in the infected cells.

Size: 5 x 20 µl

Shipping: 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.

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