Product Specification Sheet

Product Name: hTERT Antigen Cell Immortalization Kit

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 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.

Catalog Number: CILV02

Size: 5 × 20 µl

Shipping: Dry Ice

Storage and Stability: 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 (> 1 × 10^8 IFU/ml) is measured by Clontech’s Lenti-X qRT-PCR Titration Kit (Cat. # 631235). Each lot of Cell Immortalization kit is functionally tested in fibroblasts.

Restricted Use: For Research Use Only. Not for use in diagnostic or therapeutic procedures.
Protocol (CILV02)

General procedure for cell immortalization

1. Plate the target cells in one well of 6-well plate at density of 1-2 × 10^5 cells/well.
2. The next day, thaw the concentrated recombinant lentivirus in a 37° C water bath and remove it from the bath immediately when thawed.
3. Infect the target cells in a 6-well plate with 4-20 µl/well supernatant in the presence of 4 µl TransPlus reagent (ALSTEM, Cat. # V050).
   Note: TransPlus reagent is a polycation that neutralizes charge interactions to increase binding between the pseudoviral capsid and the cellular membrane.
4. The next day, remove 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 × 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: After thawing, we recommend that the supernatant not be frozen again for future use since the virus-titer will decrease significantly.