

hTERT Cell Immortalization Kit Protocol

Product Descriptions

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 are able to 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.

Protocol

1. Plate the target cells in one well of 6-well plate at density of $1-2 \times 10^5$ cells/well.
1. The next day, take one vial of the concentrated recombinant lentivirus from -80°C freezer and thaw it on ice.
2. Infect the target cells in a 6-well plate with 4-20 μl /well viral 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.
3. The next day, aspirate medium containing viral supernatant and add the appropriate complete growth medium to the cells and incubate at 37°C .
4. 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.
5. 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 (ALSTEM, cat# VF050) into the thawed supernatant before frozen again for future use.

