



Immortalization Kit

Product Information

Catalog Number	ASK-3501
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. 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. Provided with this kit is the Virus Transduction Enhancer (ASK-3501-2) which is a mixture of polymers optimized for the infection of lentivirus or retrovirus in most cells. It can increase transduction rate up to 10 fold.
Quantity	5 x 20 μ L Immortalization reagent (ASK-3501-1) 100 μ L Virus Transduction Enhancer (ASK-3501-2)
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.
Shipping	Dry Ice
Storage and Stability	Store the immortalization reagent at -80 °C (stable up to six months); store the Transduction Enhancer at 4°C for up to 6 months.
BioSafety Level	BSL-2
Restricted Use	Reagents and materials included in Immortalization Kit are intended for research purposes only and not intended for human or animal diagnostic or therapeutic uses.

Protocol

General procedure for cell immortalization

1. On Day 1, plate the target cells in one well of 6-well plate at density of $1-2 \times 10^5$ 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 μ L/well supernatant in the presence of 4 μ L 1x Viral Transduction Enhancer (ASK-3501-2).

Note: Virus Transduction Enhancer reagent is a polycation that neutralizes charge interactions to increase binding between the pseudoviral capsid and the cellular membrane. The transduction enhancer is provided as a 500x concentrated stock. Prepare a 1x working solution in culture medium before use.

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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 2x 100mm 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.

Restriction

This product is for research use only. Use in and/or for diagnostics or therapeutics is strictly prohibited. By opening and using the product, the purchaser agrees to the following: The components in this kit may not be distributed, resold, or modified for resale without prior written approval from Applied StemCell, Inc.. If you do not agree to the above conditions, please return the unopened product to Applied StemCell, Inc. within ten (10) days of receipt for a full refund. The information in this document is subject to change without notice and should not be construed as a commitment by Applied StemCell, Inc.. or its affiliated corporations. In no event shall Applied StemCell, Inc. or its affiliated corporations be liable for incidental or consequential damages in connection with or arising from the use of this manual and product. Applied StemCell, Inc. products are warranted to meet our QC testing standards at the time of shipment. Notice of problematic products must be made to Applied StemCell, Inc. within 15 days of receipt of the product. This product warranty limits Applied StemCell, Inc.'s liability to the replacement of the product only.