Cell Immortalization Reagents FAQ

Do the usual antibiotics (penicillin and streptomycin) or a fungicide (amphotericin B) usually being added to the primary epithelial cell cultures interfere with the immortalization process via SV40 large T antigen (lower somehow the transfection efficiency)? Must we remove the above compounds before immortalization with a retroviral or a lentiviral vector?

Normally all the routine antibiotics do not interfere the infection efficiency of lenti/retro viral vectors.

What is the cell density that the customer should use for lentivirus and adenovirus infection?

Lentivirus 20-30%
Adenovirus 70%

Can you summarize the cell immortalization process?

It is important to have a control when you perform the immortalization experiment. This means you should seed at least two wells in the 6 well dish.

One well will be used for viral transduction and the other will remain un-transduced. If there is no selection marker, you will have to compare the growth rates between the transduced and non-transduced as well as whether the transduced cells go pass their normal passage of senescence (easier to note if they have a control side by side when passaging).

We suggest to use 1-2mL of viral supernatant to start in the presence of polybrene (at a final concentration of 2-10 ug/ml) directly into the 6-well plate. If there is a strong toxic effect on the cells, you can consider adding fresh media but note that this will dilute the viral concentration.

You do not have to passage after 48/72 hours if the cells in the 6 well has not reached confluency. After you have passaged to 100mm dishes, keep passaging until the cells go beyond their passaging limit. From there, you can pick monoclones and check insert expression using Western Blotting, PCR, qPCR,etc.

How many rounds of immortalization will 10ml of viral reagent give me?

This will be entirely depended on your experimental parameters (cell type, seeding density etc.) and therefore cannot be determined. We would advise carrying out a pilot experiment with a GFP control lentivirus (LV006) in order to determine an optimal MOI for your experiments before carrying out the immortalization steps.

Does lentiviral titer deteriorate with every freeze thaw?

Yes, there will be a decline in the lentiviral titer with every freeze-thaw. If you do not intend to use the full volume of virus in your first round of immortalization experiments, we advise aliquoting your stock to avoid multiple freeze thaw cycles.

Can you recommend a reagent and protocol to use for my project?

It is difficult to predict which immortalization reagent will provide the most successful clones in specific cases. Successful cell immortalization can be tricky and will need to be determined experimentally. We have experienced the most overall success in a range of mammalian cell types using the SV40 whole gene and hTERT lentiviral methods. A cell immortalization user guide is also available on our web site, containing a guideline protocol (http://www.abmgood.com/SV40-Cell-Immortalization.html). Please note, this will likely require optimization and we recommend carrying out preliminary experiments to determine the lentiviral infection efficiency of the cells using a Lenti-GFP control (LV006) before attempting the immortalization step.

How do I thaw the 10ml tube of virus supernatant?

It will be fine to thaw 10 ml lentiviral supernatant in a waterbath. This is not normally required for smaller vials as they will thaw relatively quickly once removed from the freezer.