Applied StemCell

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FAQ

iPS Cell Generation Kit

Cat. Numbers:

ASR-2000	ASR-2002	ASR-2004
ASR-2001	ASR-2003	ASK-3011

1. Do I have to determine the MOI for my transductions?

Yes, in order to achieve both a consistent transduction and reprogramming efficiency, you need to determine the efficiency that the retroviruses infect the cell. On one hand, a MOI ratio that is too low will lead to a low transduction and reprogramming efficiency (i.e. the number of clones that turn out to be actual reprogrammed iPSCs will be low). On the other hand, a MOI ratio that is too high will lead to cell fusion (and you will have to start over).

2. Can't I just use a ratio based on previous published results or transductions?

No. The ASC retroviral kit has been designed such that high transduction (20-50%) efficiencies are achieved when the MOI is determined empirically. The actual MOI ratio that will give you optimal transduction and reprogramming efficiencies will depend on many factors: Viral handling (freeze/thaw cycles), initial titer, type of cell line that will be transduced/reprogrammed, age of the cells at the time of transduction (for optimal reprogramming efficiencies use cell lines with a lower passage number as possible). SO it is not wise to arbitrarily choose a MOI. Traditionally, MOI ratios have been used that have a significantly higher Oct4 MOI that the remaining Yamanaka factors. However, the ASC transduction kit has optimized such that the ratio of (Oct4, Sox2, Klf1 and L-myc) is closer to 1:1:1:1. Using a traditional MOI ratio will not give you optimal results.

USING AN MOI BASED ON THE TRANSDUCTION EFFICIENCY OF THE GFP CONTROL VECTOR WILL ASSURE YOU THAT YOUR TRANSDUCTIONS ARE PERFORMED USING THE BEST MOI RATIO.

3. I transduced my cell line using the optimized MOI. After the second transduction, I replated the cells on 10cm dishes. After a few days the cells stopped growing. What happened?

There are two possibilities: I) The cells might have been re-plated at too low a density. The re-plating density must give you at least 50% confluency. II) The MOI ratio calculation was off. Re-check the transduction efficiency using your cell line and the control GMP vector. Compare to a robust control line such as early passage foreskin fibroblast. The transduction efficiency should be ~20-50%. If you used a MOI based on a transduction efficiency in the lower part of the range, adjust the MOI based on a transduction efficiency at the higher end of the range.

4. How long will the viruses remain stable?

The viruses should remain stable for 6-8 months if kept at -80^{o}C. However, several precautions should be taken: 1) Freeze/thaw the viruses ONCE. When ready, thaw the viruses on ice. Closely monitor when complete melting is achieved. While on ice, aliquot the viruses using appropriate volumes based on your initial calculations. Freeze all aliquots at -80^{o}C. When using an aliquot for a transduction, thaw the vial in ice. Once done with the day-1 transduction. **DO NOT RE-FREEZE THE VIRUSES. STORE THE VIRUSES AT 4^{o}C UNTIL THE SECOND TRANSDUCTION. AT THAT TIME PLACE THE VIRUSES ON ICE AND PROCEED.**

5. Q: How long does it take before I see iPSC colonies?

Normally, the first iPSC colonies should appear around 13-18 days after the second round of transduction and the subsequent re-plating. However, the appearance of iPSC colonies depends on several factors:

I) The MOI used to transduce the cell line (a suboptimal MOI ratio of factors will yield lower iPSC colonies and/or longer time for the iPSC colonies to appear).

II) “Fitness” of the cell line. Older cell lines and cell lines with a higher passage number will not yield as much iPSC colonies as younger cell lines or cell lines at a lower passage number. Also, cell lines derived from individuals with severe genetic characteristics (i.e. a very old person that is homozygous for a disease gene) tend to yield lower iPSC colonies than heterozygous or younger counterparts.

III) Partially differentiated or transformed cells can take over a plate and overwhelm any potential iPSC colonies. This is sometimes seen in transductions involving c-myc as one of the reprogramming vectors. Using the L-myc viral vector should decrease the odds of this. Partially differentiated or transformed cells can also occur when the MOI is not optimal. At this point it is best to remove any potential iPSC as fast as possible and closely monitor the plate.

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6. I picked some tentative iPSC clones from my initial screening. Some of these clones either differentiated or died off in subsequent passages. Is this normal?

Yes. There are many instances were an iPSC clone candidate will either differentiate or die off. This is why it is important to pick as many potential iPSC candidates as possible.

7. Is polybrene necessary for the transductions?

Yes, polybrene is necessary to achieve optimal transduction efficiency.

8. Are two rounds of transduction necessary?

A: Traditionally, two rounds of transduction have been shown to give optimal results. Sometimes a particular cell line will exhibit toxic effects when transduced twice. To solve this problem, try the transductions using a lower MOI.

9. When can I take the transduced cells out from the BSL-2 lab?

After the second transduction is completed, the virus-containing media is removed, the wells are washed with reprogramming media and the cells are incubated in fresh reprogramming media. At this point, the cells can be transferred to a BSL-1 lab without any fear of viral contamination.

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