VALIDATED LENTIVIRAL SHRNA

DESCRIPTION

Short hairpin RNA (shRNA) is a powerful tool to silence target gene expression through RNA interference. The lentiviral shRNA vector enables stable integration into a cell's genome to provide a more complete knockdown. It is also particularly powerful for difficult-to-target and non-dividing cells. At GenuIN Biotech, we have developed a proprietary shRNA-mediated gene knockdown platform that allows for easy stable cell line generation.

KEY FEATURES

- **100% guaranteed.** We validated the knockdown in house using Western blotting, immunocytochemistry, or flow cytometry to provide assurance that your gene will be knocked down using our product.
- **As easy as 1-2-3.** Generating stable cell lines with this gene knockdown approach can be quite easy. Just follow these steps to generate your stable cell line.
- **Efficient knockdown for most cell types.** Whether your cells are dividing or terminally differentiated, easy to transfect or hard to manipulate, the lentivirus can overcome most of these problems.
- **Save your time and money.** Stop doing trial and error without knowing whether the design will work. We do this for you so you can focus on more important things.

PROTOCOL

1. Lentiviral shRNA in growth medium will be shipped to you on dry ice via an overnight carrier.
2. Upon arrival, store the product at -80°C until use.
3. Culture the human cell line of your choice in a 6-well plate in 2 mL of growth medium until cells reach 80% confluence.
4. After reaching 80% confluency, prepare the lentiviral medium by pre-warming to 37°C. Discard 1mL of growth medium from the well and add 1mL of prewarmed lentiviral medium. Gently swirl to mix. Note: The volume of medium in the well should now be 2mL.
5. Add the provided polybrene to a final concentration of 5 µg/mL, mix well by swirling.
6. Culture the cells in 37°C incubator. Store the remaining 1 mL of viral medium at -80°C.
7. The following day, prewarm the remaining 1 mL of viral medium to 37°C and add it to the growing cells. Note: The medium volume should now be 3 mL. Also, add extra polybrene into the medium to reach a final concentration of 5 µg/mL. *Tip: calculate polybrene concentration based on the additional 1 mL of the medium because the original medium already contains 5 µg/mL polybrene.*
8. The following day, discard the medium into bleach (10% final concentration) and add fresh growth medium containing 4 µg/mL puromycin to kill the cells that have not been infected by the shRNA viruses. *Tip: different cell lines have different tolerances for puromycin, therefore, it is strongly recommended to titrate the optimal puromycin concentration and kill time to find an appropriate concentration-time combination. It is*

also recommended to always include a control well of cells that have not been infected during the puromycin selection to ensure the selection was successful.

9. 48 h after the addition of puromycin, change the medium to normal growth medium. *Tip: 48h is generally sufficient for most mammalian cells to die in 4 µg/mL puromycin. However, titration is recommended to find the optimal concentration-time combination.*
10. Allow the cells to recover and grow in normal medium until they reach 90% confluence.
11. You can split the cells into more plates, freeze the cells, and/or lyse the cells for desired experiments.