Protocol

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Noncovalent Protein Internalization into Mammalian Cells

Introduction

Introduction of active and properly functioning proteins into mammalian cells has proved a useful tool in the treatment of various diseases. It is now known that proteins can be delivered into both mammalian and plant cells using arginine-rich intracellular delivery (AID) peptides in a noncovalent manner. This technique allows for the quick and simple internalization of functioning proteins into cells. This protocol outlines the use of arginine-rich intracellular delivery (AID) peptides to deliver fluorescent proteins or β-galactosidase enzymes into animal or plant cells, providing a useful strategy to introduce active proteins in cells and tissues *in vivo*. In this protocol, X-Gal is used to provide a visual indicator since it can be hydrolyzed by β-galactosidase, yielding 5,5'-dibromo-4,4'-dichloro-indigo-2, a blue product.

Materials

- β-Gal protein
- AID peptides in PBS
- MCF7 cells
- PBS (Phosphate-Buffered Saline) (GoldBio Catalog # P-271)
- X-Gal (GoldBio Catalog # X4281C)

Method

In this procedure, cells are treated with β-Gal (control) or β-Gal/AID (experimental), followed by X-Gal treatment. After washing the X-gal away, cells can then be observed under a microscope.

1. For the control sample, aspirate medium and treat cells with 0.5µM β-Gal (5 µg β-Gal in a sample volume of 70.8 µl) for 15 minutes at room temperature.

2. For experimental samples, prepare a solution containing 0.5µM β-Gal and 12.1µM AID peptides (1:24 ratio), vortex for 10 seconds and incubate at room temperature for 20 minutes. Treat experimental cells with this β-Gal/AID solution at room temperature for 15 minutes.

3. Aspirate the β-Gal or β-Gal/AID solution from the control or experimental samples, respectively, and wash with 1 ml PBS three times.

4. Treat cells with 2 mg X-Gal in 1 ml PBS and incubate at 37°C for 8 hours.

5. Aspirate the X-Gal solution and wash cells with 1 ml PBS three times.

6. Observe cells under microscope. Determine internalization by localizing blue product in cells as shown below.

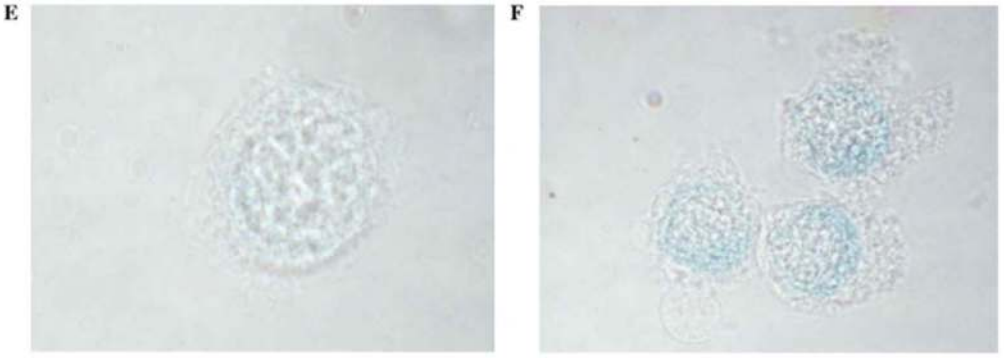

Fig. 2. Confocal microscopy of noncovalent protein internalization via AID peptides in animal cells. (E) Image of cells incubated with b-Gal in bright field. Cells were treated with 0.5μM b-Gal followed by X-gal treatment. Image was shown at a magnification of 1000x. (F) Image of cells treated with R9-plus-b-Gal mixtures in bright field. Cells were treated with 0.5μM b-Gal pre-mixed with 12.1μM of the R9 peptide followed by X-gal treatment.

Tips

- MCF7 cells are maintained in RPMI 1640 medium supplemented with 10% heat-inactivated bovine serum and 1X penicillin/streptomycin/amphotericin B.

Associated Products

- PBS (GoldBio Catalog # P-271)
- X-Gal (GoldBio Catalog # X4281C)

References

Wang, Y. H., Chen, C. P., Chan, M. H., Chang, M., Hou, Y. W., Chen, H. H., Hsu, H.R., Liu, K., and Lee, H. J. (2006). Arginine-rich intracellular delivery peptides noncovalently transport protein into living cells. *Biochemical and biophysical research communications*, 346(3), 758-767.