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Protocol: Transducing Target Cells with AAV

Materials

- AAV vector stock
- Target cells (adherent or suspension)
- Appropriate cell culture medium
- Polybrene (optional for enhancing transduction efficiency)
- Phosphate-buffered saline (PBS)
- Trypsin-EDTA (for adherent cells)
- Cell culture plates or flasks

Equipment

- Incubator set to appropriate conditions (37°C, 5% CO_2)
- Centrifuge
- Hemocytometer or cell counter
- Inverted microscope
- Pipettes and sterile tips
- Biosafety cabinet

Protocol Steps

Recombinant adeno-associated virus vectors, though replication-deficient, transduce mammalian cells and should be handled with BSL-2 standards.

1. **Cell Preparation**

1.1. Seed target cells in appropriate culture vessels (plates or flasks) and grow until they reach the desired confluency (typically 70-80% for adherent cells).

1.2. For suspension cells, ensure they are in the exponential growth phase.

2. **AAV Vector Preparation**

2.1. Thaw AAV vector stock on ice if frozen. Mix gently by pipetting up and down. Avoid repeated freeze-thaw cycles.

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2.2. Determine the desired multiplicity of infection (MOI), the ratio of viral particles to target cells. This can vary based on the cell type and the specific application (common MOIs range from 10^3 to 10^6 TU/cell).

3. **Transduction**

3.1. For adherent cells: Remove the culture medium and replace it with a fresh medium containing the AAV vector at the desired MOI. If using polybrene (e.g., at 4-8 µg/mL), add it to the medium to enhance transduction efficiency.

3.2. For suspension cells: Centrifuge the cells and resuspend them in the medium containing AAV at the desired MOI. Polybrene can also be added here if applicable.

3.3. Gently swirl the culture vessel to ensure even distribution of the vector.

4. **Incubation**

4.1. Incubate the cells with the AAV vector at the appropriate temperature (usually 37°C) and CO_2 conditions for 24-48 hours.

4.2. For adherent cells, gently rock the culture vessel a few times during incubation to ensure an even distribution of the vector.

5. **Post-Transduction**

5.1. After incubation, remove the transduction medium and replace it with a fresh culture medium.

5.2. Continue to culture the cells under standard conditions for the desired time before analyzing gene expression or phenotypic changes.

6. **Analysis**

6.1. Depending on the experimental design, assess transduction efficiency and gene expression using appropriate methods (e.g., fluorescence microscopy for GFP, flow cytometry, qPCR, Western blotting).

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Tips for Optimization

- **MOI Optimization**: Test different MOIs to find the optimal one for your cell type and application.
- **Cell Density**: To maximize transduction efficiency, ensure cells are healthy and at the right confluency.
- **Polymer Additives**: Polybrene or other transduction enhancers can improve efficiency but should be tested for toxicity in your specific cell line.

7. Troubleshooting

7.1 Improper MOI

Solution: Optimize MOI.

7.2 Low Transduction Efficiency

Solution: Verify the AAV serotype suitability for the target tissue.