ADENOVIRUS INFECTION PROTOCOL

User Manual:
Ready-to-Use Recombinant Adenovirus

- Storage, Handling, and Infection Protocol

SignaGen® Laboratories
5260 Westview Dr, Suite 200
Frederick MD 21703
FAX. 301-560-4919
TEL. 301-330-5966
Email: info@signagen.com
Web: www.signagen.com

This product is for laboratory research ONLY and not for diagnostic use

Product Overview:
Recombinant adenovirus enables efficient gene delivery into a wide range of mammalian cell types and offers the following advantages:

- High transduction efficiency, often approaching near-complete infection in permissive cell types
- Compatibility with serum-containing or serum-free culture conditions
- Direct application to cells without the need for transduction enhancers
- Flexible infection duration, with optional media replacement post-infection

In Vitro Infection Protocol:

1. Preparation of Virus-Containing Medium
Thaw adenoviral stock on ice or at room temperature.
Dilute the virus to the desired concentration in appropriate culture medium (e.g., DMEM or cell-specific medium).
Prepare sufficient volume for the intended culture format.

2. Infection of Mammalian Cells

- Remove existing culture medium from cells.
- Add virus-containing medium according to the culture vessel:

Culture Format	Recommended Volume
24-well plate	~ 0.5 mL
12-well plate	~ 1.0 mL
6-well plate	~ 2.0 mL
60 mm dish	~ 6.0 mL
10 cm dish	~ 15 mL

- Incubate cells with virus for 12 ~ 18 hours or longer, depending on experimental design.
- (Optional) Replace with fresh medium after infection.
- Check transgene expression at 24 ~ 72 hours post-infection in most cell types. Peak expression is typically observed at approximately 72 hours post-infection in mammalian cells.

3. Optimization of Viral Dose (MOI)
Efficient adenoviral transduction depends on cell type and receptor expression levels.

- Typical multiplicity of infection (MOI): **10–300** for most adherent cell lines
- Higher MOI (up to **~3000**) may be required for difficult-to-transduce cells (e.g., lymphoid cells)
- Excessive MOI may induce cytotoxicity

Recommendation:
Perform pilot experiments using a reporter virus (e.g., GFP-expressing adenovirus, SKU # SL100708) to determine optimal MOI for your specific cell type.

Storage and Handling:
Recombinant adenovirus is supplied as a liquid preparation at the indicated titer in DMEM supplemented with 2.5% glycerol.

- Store immediately at –80 °C upon receipt.
- Aliquot viral stocks as needed to avoid repeated freeze–thaw cycles.
- Avoid multiple freeze–thaw cycles, as this significantly reduces viral infectivity.

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In Vivo Injection Protocol (Mouse Tail Vein Delivery):
Intravenous administration via the tail vein is a widely used method for liver-directed adenoviral gene delivery in mice. This approach avoids surgical procedures and provides efficient systemic distribution.

Materials:
- Adult mice (>6 weeks old, 20 ~ 25 g)
- Recombinant adenovirus: 2×10^9 – 1×10^{10} PFU per mouse
- Sterile PBS or DMEM
- 0.5 mL syringe with **27G needle (½ inch)**
- 70% ethanol
- Mouse restrainer
- Heat source (lamp or warm water)
- Gauze pads

Injection Procedure:
1. **Vasodilation**
- Warm mice under a heat lamp (15 ~ 25 cm distance) for ~5 minutes to dilate tail veins.
- Alternatively, immerse the tail in warm water until the veins are visible.
2. **Preparation**
- Restrain the mouse.
- Clean the tail with 70% ethanol.
3. **Virus Preparation**
- Load **~0.1 mL** adenovirus solution (2×10^{10} – 1×10^{11} PFU/mL).
- Remove air bubbles from the syringe.
4. **Injection**
- Insert the needle into the lateral tail vein at a shallow angle.
- Inject slowly (~0.1 mL total volume).

Indicators of correct injection:
- Vein becomes transiently clear
- No resistance during injection

If swelling occurs:
- Stop immediately (indicates subcutaneous injection)

5. **Post-Injection Care**
- Withdraw the needle and apply pressure until bleeding stops.
- Return the mouse to the cage and monitor.
6. **Assessment**
- Evaluate transgene expression at 4 ~8 days post injection.

Notes and Considerations:
Injection via the **portal vein** is not typically recommended due to surgical requirements and limited efficiency gains.
Tail vein injection provides a balance of efficiency, reproducibility, and minimal invasiveness.
Viral dose, purity, and animal strain may influence transduction outcomes.

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