User Manual for Adenovirus

User Manual: Ready-To-Use Recombinant Adenovirus

- Storage Instructions and Infection Protocol

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

Contents and Storage:

Recombinant adenovirus is supplied in liquid form at indicated titer. The storage solution is DMEM/2.5% glycerol. Store at -80 ^{0}C. If desired, aliquot viral stock upon arrival, and store those aliquots at -80 ^{0}C Freeze immediately. ***DO NOT FREEZE AND THAW REPEATEDLY***

Description:

Recombinant adenovirus is for delivering interested genes into mammalian cells. It provides the following advantages:

1) 100% efficiency of gene delivery in many cell types.
2) Recombinant viruses can be added directly to cells in culture medium (in the presence or absence of serum).
3) It is not necessary to remove viruses, change or add medium following infection, although viruses can be removed after 6-12 hours post infections.

In-vitro Infection Protocol:

1. Prepare virus-containing media:

Thaw viral stock at either room temperature or on ice.
Add desired amount of virus to media. If needed, viruses could be diluted further in DMEM or other media

2. Infecting cells with adenovirus:

Remove the original cell culture media, and add the above virus-containing media to cell culture. Below is a general guideline for the amount of media used:

24-well plate:	0.2-0.3 mL
12-well plate:	0.5-0.8 mL
6-well plate:	1-1.5 mL/well
60mm-plate:	3-4 mL/plate
10cm-plate:	8-12 mL/plate

Incubate cells with the virus-containing media for 6-12 hours, or as long as you wish.
(Optional), you could remove virus-containing media and replace it with fresh, desired media.

The appropriate amount of viruses used for infecting cells is critical for the outcome of your experiments. The goal is to get 100% of infection without causing cytotoxicity or other undesired effects. The amount of adenovirus cell surface receptors vary greatly among different cell types therefore the optimal concentration differs dramatically between cell types. A range of 10-100 MOI (multiplicity of infection) is used for most cell lines, but up to 1000 MOI may be used for lymphoid cell lines.

To determine this optimal concentration of virus for your study, you could conduct pilot testing in your cell line by using reporter adenoviruses, such as Ad-CMV-GFP (Cat. # SL100708).

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In-Vivo Injection Protocol:
A brief protocol to inject adenovirus to mouse via tail vein

Livers can be transduced with recombinant adenovirus by intravenous injections through peripheral veins. For mice, tail vein injections are generally preferred, as they require neither surgery nor anesthesia. Alternative sites of injection include the jugular vein. Portal vein injections require surgery and recovery time, and are not significantly more efficient for delivery of adenovirus to the liver, thus they are generally not used.

Materials:

- Adult mice, at least 6 weeks of age, 20 to 25 g.
- 70% ethanol in squirt bottle.
- Total 2~10E+9 PFU purified recombinant adenovirus vector per mouse in sterile PBS or DMEM via tail vein.
- Mouse cage with wire lid.
- Heat lamp.
- Mouse restrainer.
- Cotton gauze pads.
- 0.5 mL syringe with 27-G (1/2-in.) needle

Injection Procedures:

1. Place mice to be injected (up to 6 mice) in cage and shine a heat lamp ~6 to 10 in. (15 to 25 cm) above the cage through the wire lid to warm them up.
   Watch the mice for ~5 min, they will be huddled together in a corner of the cage and will be ready to inject. Do not continue to actively heat the mice at this point.
   Note: The heating will cause dilation of the tail vein and therefore make the mice significantly easier to inject. Alternatively place mouse into a restrainer and dip tail in warm water for several minutes until the tail vein dilates. This will take longer, as mice have to be handled one by one.
2. Place one mouse into a restrainer. Apply 70% ethanol to the tail and wipe off excess with gauze pad.
3. Draw at least 0.1 mL of super purified adenovirus particles (in vivo grade) at 2E+10~1E+11 PFU/mL (total 2~10E+9 PFU) into a 0.5-mL syringe with a 27-G needle and carefully remove air from the needle.
4. Hold the end of the tail so that it is extended and twist it slightly to one side to see the vein. Insert the needle at a shallow angle (as the tail vein is relatively close to the surface) and inject 0.1 ml of diluted recombinant adenovirus into the tail vein.
   If the needle is correctly positioned in the tail vein, the vein will transiently shift to a clear color, and the injection should proceed smoothly. If any local swelling is observed, stop the injection, as in that case the virus is being injected into the tail tissue, not the vein.
5. Withdraw the needle and immediately apply pressure with gloved finger until bleeding stops.
6. Return mouse to original cage.
7. Check the transduction efficiency.

For further tech inquires, please email us at info@signagen.com