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User Manual for Ready-To-Use Recombinant Adenovirus

CONTENTS AND STORAGE

Recombinant adenovirus is supplied in liquid form at indicated titer. The storage solution is DMEM/2.5% glycerol. Store at -80°C. If desired, aliquot viral stock upon arrival, and store those aliquots at -80°C freezer 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.

IMPORTANT GUIDELINES

Follow these guidelines when performing infections:

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

PFU needed = MOI (multiplicity of infection) * # of cells to be infected
e.g. If you intend to infect 1 million cells using MOI of 100 , you need 100 x 1,000,000 = 10^8 PFU for the infection. If the original stock is 10^{10} PFU/ml , then you will need 10 ul of the original stock for the dilution.

2. **Infecting cells with virus**:
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 20-200 MOI (multiplicity of infection) is used for most cell lines, but up to 4000 MOI may be used for some cell lines or primary cells.

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 GFP adenovirus (Cat.#1060).

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