# Protocol for Adenoviral Transduction of Human Cells

cells in focus



## 1. General Information

This protocol describes the standard technique for handling recombinant adenoviruses, as well as instructions on how to design an approach for transducing human cells. As an example, a transduction experiment of primary Human Umbilical Vein Endothelial Cells (HUVEC) with a recombinant adenovirus harboring LifeAct®-TagGFP2 transgene is described.

## 2. Background

Replication-deficient recombinant adenoviruses (serotype 5) are widely used in research laboratories. This modified adenovirus, where genes E1 and E3 have been depleted, is still able to infect cells. However, the essential genes for producing new viral particles, also known as virions, are no longer present.

There are numerous advantages to use an adenovirus for introducing genetic material into host cells. These viruses can be used to transduce many mammalian (especially human) dividing and non-dividing cell types, both *in vitro* and *in vivo*. Moreover, recombinant adenoviruses can be used to transduce various sensitive cells.

The attachment of adenoviruses to cells is mediated by high-affinity binding to the Coxsackie-Adenovirus Receptor (CAR), while internalization occurs through

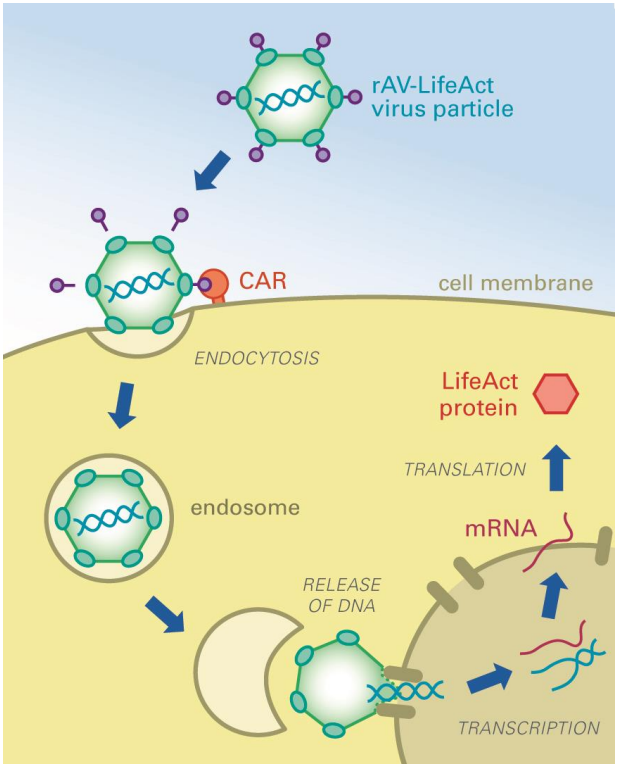


**Figure 1: Simplified illustration of the adenoviral transduction mechanism.**

endocytosis upon interaction with αV-integrins. By means of transport mechanisms provided by microtubules, the adenovirus reaches the host cell nucleus and injects its DNA into it.

After entering the nucleus, the viral DNA remains epichromosomal (i.e., it does not integrate into the host chromosome and therefore does not activate or inactivate host genes). A simplified illustration of the infection mechanism is shown in Figure 1.

## 3. Material and Equipment Required

For this protocol, the following materials are required:

**Table 1: Materials needed for the transduction of HUVEC.**

| Name | Company | Order No. |
|---|---|---|
| rAV$^{CMV}$-LifeAct®-TagGFP2 | ibidi GmbH | 60121 |
| µ-Slide 8 Well ibiTreat | ibidi GmbH | 80826 |
| HUVEC | commercially available | - |
| Endothelial Cell Growth Medium incl. ECGM supplement | Promocell GmbH | C-22010 |
| Penicillin/Streptomycin | Sigma-Aldrich Chemie GmbH | P4333 |
| ibiBoost™ Adenovirus Transduction Enhancer | ibidi GmbH | 50301 |

For this protocol the following equipment and instruments are required:

- Cell culture incubator (high humidity, 37°C, 5% $CO_2$)
- Class II biological safety cabinet
- Fluorescence microscope equipped with an appropriate filter set for TagGFP2 (fluorescence properties: $Ex_{max}$ 483 nm / $Em_{max}$ 506 nm), a stage top incubator (37°C, 5% CO2), and an optional time lapse function

## 4. Safety and Handling of Recombinant Adenoviruses

Avoid repeated thawing and freezing cycles during the performance of the experiments as this leads to a strong decrease in viral titer. Thawing should occur on ice, and the adenovirus should be used directly afterwards. Freezing should occur either on dry ice or at a temperature of -80°C.

In addition, to ensure that the quality of the virus is maintained, we recommend aliquoting the vial contents on first use.

Keep in mind that you will be working with infectious viral particles. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. The use of a Class II biological safety cabinet is mandatory. Work under a biosafety hood, use filtered tips, and wear gloves.

# 5. Transduction of HUVEC with rAV$^{CMV}$-LifeAct®-TagGFP2

## Basic terms

MOI: Multiplicity of Infection

IU: Infectious Unit (also known as IFU)

rAV: recombinant Adenovirus

Experiments can be started once the MOI, one that is most suitable for the cells of interest, has been determined (see Section 7 for further information). In this example, HUVEC cultured in a µ-Slide 8 Well ibiTreat have been used. As shown below, these cells can be efficiently transduced with a MOI of 100.

### a. Calculating the amount of virus required in a µ-Slide 8 Well

General formulas:

I. $\text{virus needed}[\text{IU}] = \text{cell number seeded} * \text{MOI}$

II. $\frac{\text{virus needed}[\text{IU}]}{\text{viral titer}[\text{IU}/\mu\text{l}]} = \mu\text{l needed}$

Example:

I. Amount of virus needed for transduction:

   Cell number seeded = 1.2x $10^5$ cells per well; MOI = 100

   ⇨ Virus needed [IU]: 1.2x $10^5$ (cells) * (MOI) 100 = 12x $10^6$ IU per well

II. Transforming the amount of virus into µl:

   Viral titer = 1x $10^{10}$ IU/ml = 1x $10^7$ IU/µl; Virus needed [IU] = 12x $10^6$ IU

   ⇨ Virus needed [µl]: 12x $10^6$ IU / 1x $10^7$ IU/µl = 1.2 µl viral stock per well

As a result, 1.2 µl of viral stock (i.e., 1x $10^{10}$ IU/ml) is needed to transduce 1.2x $10^5$ cells with a MOI of 100.

**Table 2: Number of cells and number of infectious adenoviral particles needed for setting up an adenoviral experiment with HUVEC in other formats.**

| | | | Adenovirus Amount [µl] | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Format** | **HUVEC /Well** | **Volume /Well** | **MOI 10** | **MOI 40** | **MOI 100** | **MOI 200** | **MOI 500** | **MOI 1000** |
| 48 well | 5x $10^4$ | 250 µl | 0.05 | 0.2 | 0.5 | 1 | 2.5 | 5 |
| 24 well | 1x $10^5$ | 500 µl | 0.1 | 0.4 | 1 | 2 | 5 | 10 |
| 12 well | 2x $10^5$ | 1 ml | 0.2 | 0.8 | 2 | 4 | 10 | 20 |
| 6 well | 4x $10^5$ | 2 ml | 0.4 | 1.6 | 4 | 8 | 20 | 40 |
| µ-Slide 8 Well | 1.2x $10^5$ | 300 µl | 0.12 | 0.48 | 1.2 | 2.4 | 6 | 12 |

### b. Seeding cells

The day before the transduction, seed 1.2x $10^5$ HUVEC in 300 µl of endothelial cell growth medium per well of a µ-Slide 8 Well. The cells should exhibit 50-60% confluence at the time of transduction.

### c. Transduction

Note: If a cell type is transduced with an adenovirus for the first time it is recommended to perform a setup with different MOI using an adenovirus encoding a fluorescent protein. (see Table 2)

1. Thaw the adenovirus on ice.
2. Mix 0.8 µl of ibiBoost™ Adenovirus Transduction Enhancer with 150 µl of culture medium.
3. Add 1.2 µl of adenoviral particles and mix the solution by flicking the tube.
4. Incubate for 30 minutes at room temperature and 400 rpm on a shaker.
5. Remove culture medium from the cells, and add the pre-incubated Adenovirus/ibiBoost™ mixture.
6. Incubate cells for 4 hours at standard cell culture conditions and exchange Adenovirus/ibiBoost™ mixture by fresh culture medium.
7. Incubate cells at standard cell culture conditions. The LifeAct®-TagGFP2 signal is visible after 1-2 days.
8. Image cells by fluorescence microscopy or perform another assay (e.g., flow cytometry).

### d. Results

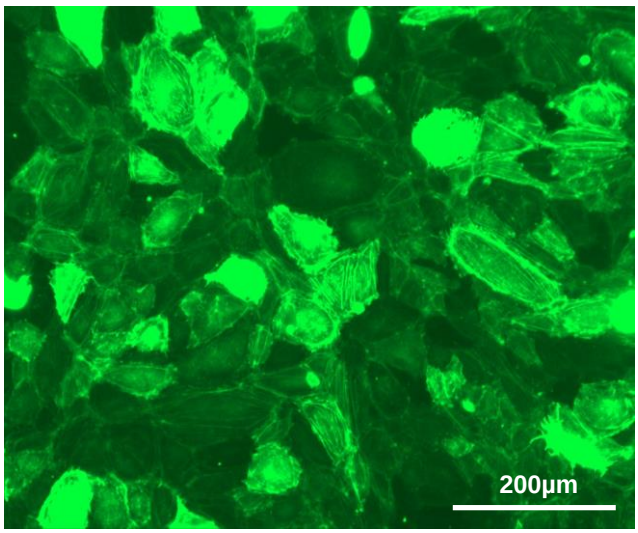


**Figure 2: Fluorescence images of living HUVEC (Passage 1, confluence 100%) transduced with the rAV$^{CMV}$-LifeAct®-TagGFP2 and a MOI of 100. The images were taken 48 hours after transduction.**

Figure 2 shows HUVEC transduced with a MOI of 100 and imaged 48 hours after transduction. Almost 100% of the cells are expressing LifeAct®-TagGFP2, which causes the bright staining of the actin cytoskeleton.

## 6. Troubleshooting

| Problem | Reason | Solution |
|---|---|---|
| **Low transduction efficiency** | MOI used is too low. | Use higher amount of adenovirus. |
| | Viral titer is not the infectious titer. | Please follow the instructions of chapter 7 “Determining the MOI”. |
| | Cells are very hard to transduce. | Increase the amount of adenovirus.<br>Check if cell density was too high. |
| | Incubation time on the cells was not long enough. | If cells will not suffer incubate cells overnight with the Adenovirus/ibiBoost™ mixture. |
| **Low viability** | Cells are sensitive to ibiBoost™. | Decrease the incubation time. |
| | Cells are sensitive to adenoviral treatment. | Reduce MOI. |
| | Cells are sensitive to a low amount of medium. | Prepare the Adenovirus/ibiBoostTM mixture in 1/10 volume and add it directly to the cells without removing culture medium. |

## 7. Optional: Determining the MOI

The MOI describes the number of virus particles needed to infect one cell. However, the probability of a cell infection is subject to the statistical Poisson distribution. For example, a MOI of 100 signifies that 100 virus particles are needed for one cell to be infected efficiently. In principle, the MOI for adenoviral particles ranges from 10 to 1000.

General formula:

$$\mathrm{MOI} = \frac{\mathrm{volume(virus)} * \mathrm{concentration(virus)}}{\mathrm{volume(cells)} * \mathrm{concentration(cells)}}$$

The MOI differs greatly between various cell types. Therefore, when transducing cells for the first time, we recommend determining the MOI necessary for efficient transgene expression in the cells of interest before starting with specific approaches. Various recombinant viruses can be used for this purpose, such as adenoviruses that only harbor GFP (Green Fluorescent Protein) and those that harbor β-lactamase, as they can be easily quantified.

<u>Experiment in a 24-well plate format:</u>

1. One day prior to the transduction, seed the cells of interest into seven wells (e.g., 1x $10^5$ cells per well), so that their confluence at the time of transduction is about 50-60%.
2. The next day, thaw the virus particles on ice and add them to the wells, referring to the numbers in Table 2. To establish a control, leave one well free of virus particles. For easier handling, the virus can be diluted with 1x PBS or medium and pipetted in higher quantities into the wells. The quantity of the required diluted virus will depend on the level of dilution.
3. Incubate the cells for 48 hours at 37°C under standard cell culture conditions.
4. Acquire pictures of the cells by fluorescence microscopy after 24 and 48 hours.

Analysis:

5. Determine the rate of transduced cells per well for each MOI and at each time point.
6. The lowest MOI at which all cells (or enough cells for the specific approach) show transgene expression is then used for further experimentation.

Note: High quantities of the virus could also lead to cytotoxic side effects. Therefore, in some cases it may be better to choose a lower MOI, so as to avoid artifacts due to non-healthy cells.

7. If needed, the MOI can be adjusted and further refined.

Figure 3 below illustrates possible results from testing different MOI on the cells of interest. In this example, an MOI of 500 was necessary to efficiently transduce 100% of the cells.

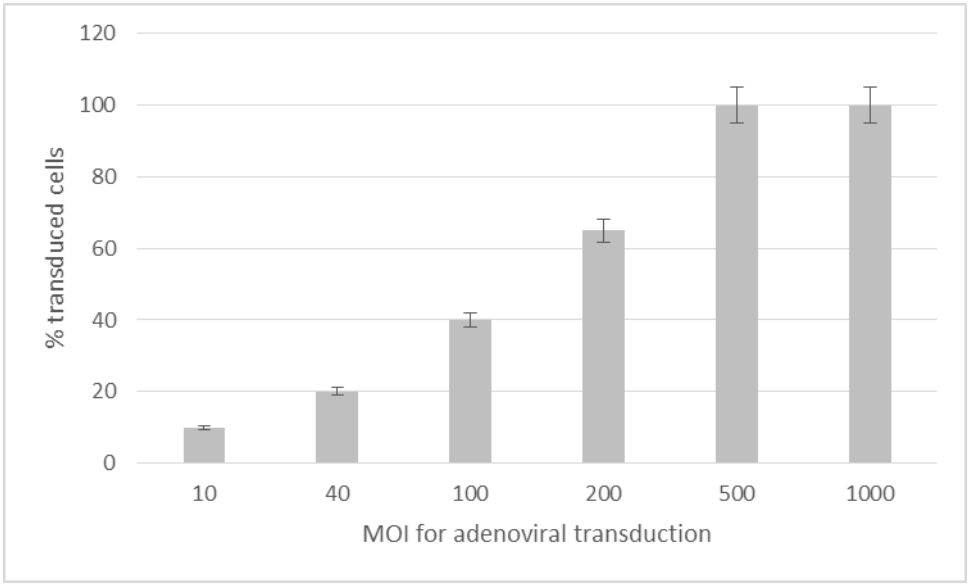


**Figure 3: Percentage of transduced cells after transduction with different MOI.**