

**Protocol for adenoviral transduction of human cells**

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**1. General Information**

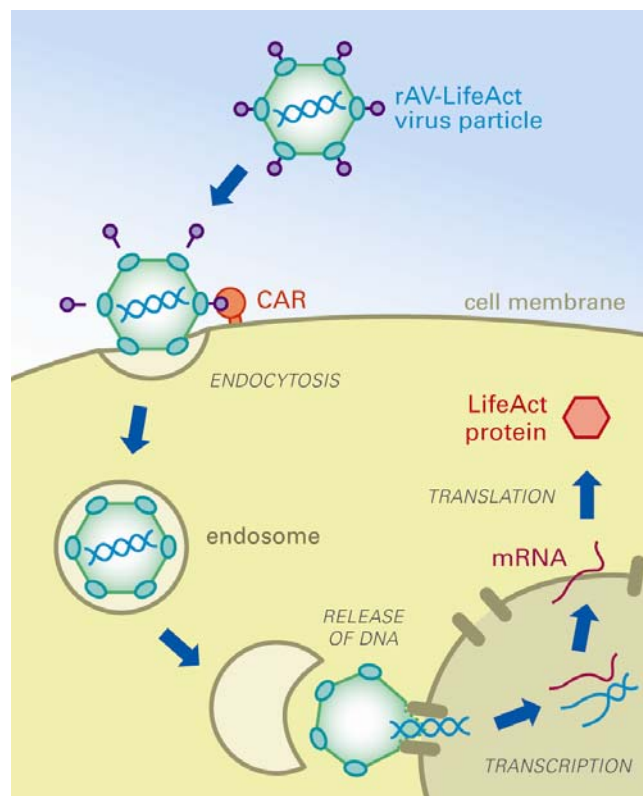
This protocol describes the standard technique for handling recombinant adenoviruses as well as how to design an approach to transduce human cells. As an example, a transduction experiment of primary Human Umbilical Vein Endothelial Cells (HUVEC) with a recombinant adenovirus harboring LifeAct-TagGFP2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

**2. Background**

These days, 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 in using an adenovirus to introduce genetic material into host cells. These viruses can be used to transduce many mammalian (especially human) cell types, both replicative and non-replicative.

Moreover, recombinant adenoviruses can also be used to transduce various sensitive cells with low transfection efficiencies.



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

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The attachment of adenoviruses to cells is mediated by high-affinity binding to the Coxsackie-Adenovirus Receptor (CAR), while internalization occurs through endocytosis upon interaction with  $\alpha$ v-integrins. By means of transport mechanisms provided by microtubules, the adenovirus reaches the host cell's 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:

	Name	Concentration	Company	Order No.
<b>Reagent/Material</b>	rAV <sup>CMV</sup> -LifeAct-TagGFP2	1x10 <sup>10</sup> IU/ml	ibidi	60121
	$\mu$ -Slide 8 well (ibiTreat)	-	ibidi	80826
<b>Cells</b>	HUVECs * (*also commercially available)	-	Self-prep <sup>†</sup>	-
	Endothelial Cell Growth Medium	1x	Promocell	C-22010
	ECGM supplement	1x	Promocell	incl.
	Penicillin/Streptomycin	5%	PAA	P11-010

**Table 1: Material and reagents needed for the transduction of HUVECs.**

For this protocol the following equipment and instruments are required:

- Cell culture incubator (high humidity, 37 °C, 5 % CO<sub>2</sub>)
- Tissue culture hood
- Fluorescence microscope equipped with a TagGFP2 filter set (Ex<sub>max</sub> 483 nm / Em<sub>max</sub> 506 nm), a stage top incubator (37 °C, 5 % CO<sub>2</sub>) and optionally, a time lapse function
- Optional, cell culture dishes (e.g., a 24 well plate)

<sup>†</sup> as published in:

Jaffe, E. A. *et al.* Culture of Human Endothelial Cells Derived from Umbilical Veins – Identification by morphologic and immunologic criteria. *Journal of Clinical Investigation* **52**, 2745-2756 (1973)

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### 4. Safety and Handling of Recombinant Adenoviruses

Recombinant viruses should not pass through more than 3 thawing and freezing cycles during the performance of the experiments. Thawing should occur on ice. 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 at its optimum, we recommend aliquoting the vial contents on first use.

Finally, keep in mind that these samples contain infectious viruses. Therefore, follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Work under a biosafety hood, use filtered tips and wear gloves.

### 5. Experiment: Transduction of HUVECs with rAV<sup>CMV</sup>-LifeAct-TagGFP2

#### Basic terms

MOI: Multiplicity of Infection

IU: Infectious Unit (also known as IFU or PFU)

rAV: recombinant Adenovirus

Experiments can be started once the MOI which is most suitable for the cells of interest has been determined (see section 6 for further information). In this example, HUVECs cultured in a  $\mu$ -Slide 8 well 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 $\mu$ -Slide 8 well

General formulas:

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

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

Amount of virus needed for transduction

Cell number seeded =  $1.2 * 10^5$  cells per well

MOI = 100

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

Transforming the amount of virus into  $\mu\text{l}$

Viral titer =  $1 * 10^{10}$  IU/ml =  $1 * 10^7$  IU/ $\mu\text{l}$

Virus needed [IU] =  $12 * 10^6$  IU

$$\Rightarrow \frac{12 * 10^6 \text{ IU}}{1 * 10^7 \text{ IU}/\mu\text{l}} = 1.2 \mu\text{l viral stock}$$

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Therefore, 1.2  $\mu\text{l}$  of viral stock (i.e.,  $1 \times 10^{10}$  IU/ml) is needed to transduce  $1.2 \times 10^5$  cells with a MOI of 100.

### b. Preparing cells for use in a $\mu$ -Slide 8 well

One day before the experiment:

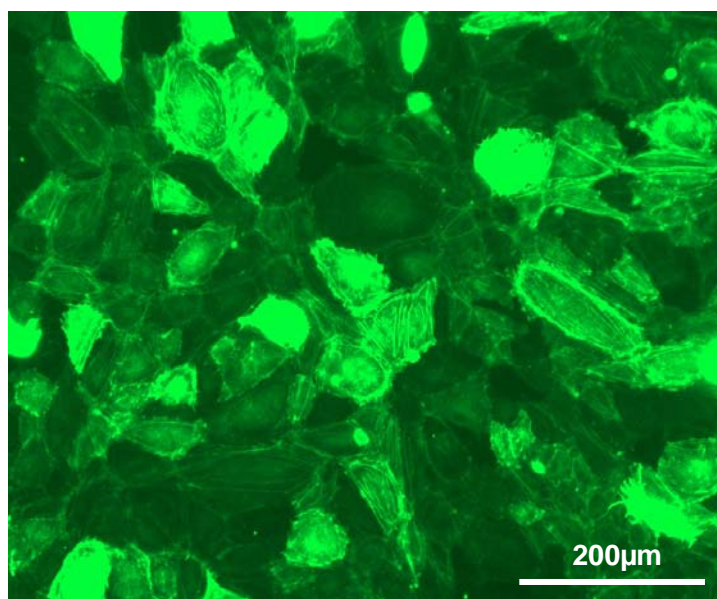
1. Prepare a cell suspension in the cell culture medium as shown in Table 1.
2. Seed  $1.2 \times 10^5$  cells per well in 300  $\mu\text{l}$  of medium.
3. Place the slide into an incubator for 24 hours under standard cell culture conditions.

The next day:

4. Exchange the medium with 300  $\mu\text{l}$  of fresh medium.
5. Thaw virus particles on ice.
6. Add 1.2  $\mu\text{l}$  of viral stock to each well.
7. Incubate cells for 24 to 48 hours at 37°C under standard cell culture conditions.
8. Image cells by fluorescence microscopy.

### c. Example results

Figure 2 below shows HUVECs (Passage 1) transduced with a MOI of 100 imaged 48 hours after transduction. Almost 100 % of the cells are expressing LifeAct-TagGFP2 leading to the bright staining of the actin cytoskeleton in the image below.



**Figure 2: HUVECs (Passage 1) transduced with a MOI of 100 and imaged 48 hours later.**

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### 6. Optional: Determining the MOI

The MOI describes the number of virus particles needed to infect one cell. However, the probability of a cell's 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 range is between 10 and 1000.

General formula:

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

The MOI differs greatly between different 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 only harboring GFP (i.e., Green Fluorescent Protein) and those harboring  $\beta$ -lactamase, as they can be easily quantified.

Experiment in a 24-well plate format:

1. One day prior to the transduction, seed the cells of interest into seven wells (e.g.,  $1 * 10^5$  cells per well) so that their confluence at the time of transduction is about 50-70%.
2. The next day, thaw the virus particles on ice and add them to the wells as per figures in Table 2. No virus particles are added to one of the wells as this well will serve as the control. For easier handling, the virus can be diluted with 1xPBS or medium and pipetted in higher quantities into the wells. The quantity of diluted virus required depends 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 that high quantities of 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 artefacts due to non-healthy cells.
7. If needed, the MOI can be adjusted and further refined.

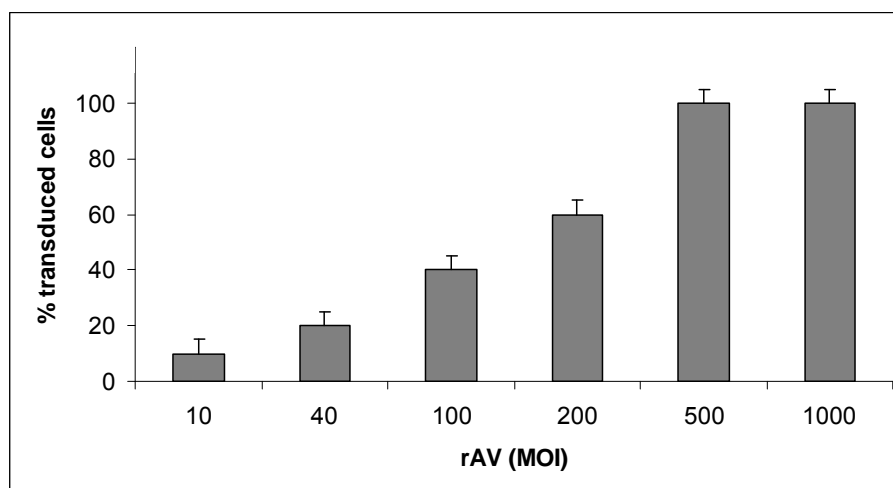
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Table 2 below provides a summary of the quantities of recombinant adenoviruses required in each well, depending on the well size chosen.

Size	Cell number	Amount of rAV ( $\mu$ l)					
		MOI 10	MOI 40	MOI 100	MOI 200	MOI 500	MOI 1000
48 well	50.000	0.05	0.2	0.5	1	2.5	5
24 well	100.000	0.1	0.4	1	2	5	10
12 well	200.000	0.2	0.8	2	4	10	20

**Table 2: Amounts of adenovirus required to determine the MOI.**

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



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