

## Tinzyme Co., Limited

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# **EZ-AAV** Transduction kit

## Product Number: TR3003

#### **Shipping and Storage**

This product is transported at room temperature and stored for a long time at 4°C, with a validity period of 12 months.

This product is safe to use and no biological or chemical toxicity has been found. If accidentally contaminated, rinse with clean water.

#### Description

EZ-AAV Transmission Kit is an enhancer developed and synthesized by our company for adeno-associated virus infection, which is synthesized using nanotechnology. The EZ-AAV Transmission kit enriches the virus on the cell surface through physical interactions to enhance infection efficiency. Due to the application of nanotechnology, the EZ-AAV Transmission kit significantly improves the efficiency of virus infection while minimizing cell toxicity and not interfering with cellular physiological functions.

### Note

- 1. After using the EZ-AAV Transmission kit for enhancement, the degree of cell fusion during infection has an impact on the infection effect. It is recommended to conduct infection experiments when the degree of cell fusion is around 50%.
- 2. Before using the EZ-AAV Transmission Kit enhancer, the optimal MOI (Multiplicity of Infection) of infected cells should be determined, which refers to the average number of viruses infected per cell. Generally, the higher the MOI, the more viruses integrate into chromosomes and the higher the surface expression of the target protein. Different cells have their optimal MOI values. We need to conduct a MOI gradient experiment first to determine the optimal MOI value for infected cells. The application of enhancers on the basis of the optimal MOI value has a better effect.
- 3. The dilution solution for EZ-AAV Transmission kit and virus is a serum-free liquid that is consistent with the cell base culture medium, such as serum-free DMEM or 1640.
- 4. The relationship between EZ-AAV Transmission Kit and infection efficiency. Under other fixed conditions, the larger the dosage of enhancers, the higher the infection efficiency. Generally, the dosage of enhancers can be used 1-3 times the recommended dosage. However, excessive use of enhancers may lead to cytotoxicity.
- 5. After using the EZ-AAV Transmission kit, sodium butyrate can also be used simultaneously.

#### Protocol

#### 1. Cell laying one day in advance

It is advisable to plant cells in advance, with a cell fusion degree of around 50% during infection (suspension cells can be adjusted as needed).

- 2. Infect
  - 2.1. Dilute the EZ-AAV Transmission kit with serum-free diluent (refer to the table below for dosage), mix thoroughly, and prepare an enhancer diluent.
  - 2.2. Dilute the virus concentrate with serum-free diluent (see table below for dosage), mix thoroughly, and prepare the virus diluent.

Note: Due to different virus concentrations, the specific amount of virus concentrate may be based on conventional dosage. It is recommended to conduct gradient experiments in combination with enhancers.

- 2.3. Mix the diluent of the enhancer and virus thoroughly, and let it stand at 4 °C for 15 minutes.
- 2.4. Add the above mixture to cells containing complete culture medium, culture at 37 °C for 8 hours, and observe the cell state. If there is no significant change, do not change the culture medium and continue to culture. Observe the expression at 24 and 48 hours.
- Table 1 Dosage of Different Cell Culture Containers

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Cell culture	Surface area	Ratio of surface	Quantity of EZ-AAV	Dosage of	Total amount of culture
container	$(cm^2)$	area to 24 well	Transmission Kit per Hole	diluent per well	medium per well
96-well	0.3	0.2	0.5-1.5µl	10µl	100µl
48-well	0.7	0.4	1-3µl	15µl	200µl
24-well	1.9	1	2.5-7.5µl	25µl	500µl
12-well	3.8	2	5-15µl	25µl	1ml
6-well/35-mm	10	5	10-30µl	50µl	2ml
60 mm/T25 flask	21	10	25-75µl	125µl	5ml
100 mm	58	30	75-225µl	250µl	15ml

2.5. Taking one well in a 6-well plate as an example, explain the amount of each liquid component used:

Enhanced	Serum-free	NAix	Concentrated	Serum-free
reagents	diluent	Mix	virus	diluent
10µl*	50µl		10µl**	50µl
		▼		

Complete culture medium per well 2000µl-60µl=1880µl

\*Can be increased or decreased separately based on factors such as toxicity and efficiency

**\*\***The amount used here is for illustration only, and the specific amount can be determined based on the regular amount at your discretion

Problem	Reason	Solution	
The effect is not significant	Low virus concentration,	Increase virus usage	
after using enhancers	insufficient dosage		
	Excessive cell density	Reduce cell density	
	The enhancement is not	Increase the dosage of enhancer by 1-3 times	
	significant		
Cytotoxicity	Excessive dosage of	Reduce the dosage of enhancers appropriately	
	enhancer		
	Excessive virus volume	Reduce virus usage appropriately	
	Less cell culture media	Increase the amount of culture medium or replace the culture	
	used	medium	
Sedimentation occurs in the	Analysis of virus	It can still be used, but pay attention to observing cell toxicity. The	
mixed solution	preservation solution	same serum-free dilution can also be added immediately to help	
		precipitate and dissolve	

## **Common problems and solutions**

## Other related reagents

TR3011: Adenovirus infection enhancing reagent.

TR3012: Chronic virus infection enhancement reagent.

TR3041: Retroviral infection enhancing reagent.

TR3015: Virus infection enhancing reagent in animal bodies.