

## Hieff Trans™ UltraAAV Transfection Reagent

### Product description

This product is a linearized polyethyleneimine (Polyethylenimine Linear, PEI) specially developed for the large-scale production of AAV in suspension culture systems. It is a chemically synthesized product, free of animal-derived components, with low cytotoxicity. The product is provided in sterile liquid form and has a wide applicability for various AAV serotypes. It features strong DNA loading capacity, low transfection complex ratio, and high complex stability, facilitating large-scale AAV production.

### Components

| Name                                       | 40823ES01-EN | 40823ES03-EN | 40823ES10-EN | 40823ES60-EN |
|--------------------------------------------|--------------|--------------|--------------|--------------|
| Hieff Trans™ UltraAAV Transfection Reagent | 100 µL       | 1 mL         | 10 mL        | 100 mL       |

### Storage

The product can be stored at 2-8°C for two years. Do not freeze!

### Instructions

#### 1. Cell Seeding

Seed cells at an appropriate density based on cell status. It is recommended to seed cells at a density of 1-1.5E6 cells/mL to achieve a cell density of 2-3E6 cells/mL on the day of transfection.

#### 2. Transfection Complex Preparation (For a 1 L system)

- 1) Plasmid to reagent ratio: The ratio of plasmid (µg) to transfection reagent (µL) is 1:0.5-1:1.
- 2) Plasmid dilution: Dilute 2 mg of plasmid using 25 mL of serum-free medium and gently mix.
- 3) Reagent dilution: Dilute 1 mL of Hieff Trans™ UltraAAV Transfection Reagent using 25 mL of serum-free medium and gently mix.
- 4) Complex formation: Add the prepared 25 mL plasmid dilution to the 25 mL transfection reagent dilution, gently vortex mix, and let it stand at room temperature for 20 minutes to form the plasmid-PEI complex, set aside.

| Parameter Type                                     | Recommended Condition | Condition Range |
|----------------------------------------------------|-----------------------|-----------------|
| Nucleic Acid Amount (per million cells)            | 1 µg                  | 0.5 µg-2 µg     |
| Nucleic Acid to Transfection Reagent Ratio (µg:µL) | 1:0.5                 | 1:0.5-1:1       |
| Transfection Complex Volume (% of culture volume)  | 5%                    | 1%-10%          |
| Complex Incubation Time                            | 20 mins               | 15 mins-60 mins |

#### 3. Transfecting Cells

- 1) Directly add 50 mL of the plasmid-PEI complex to the cells.
- 2) Continue to culture the cells at 37°C with 5% CO<sub>2</sub>, and harvest the virus after 72 hours of culture.

The amount of transfection for different cell culture volume (for reference only)

| Cell Culture Parameter |                |              | Transfection Parameter |        |                      |
|------------------------|----------------|--------------|------------------------|--------|----------------------|
| Cell Density           | Culture Volume | Total cells  | Complex volume         | DNA    | Transfection reagent |
| 2E6 cells/mL           | 30 mL          | 6E7 cells    | 1.5 mL                 | 60 µg  | 60 µL                |
|                        | 1 L            | 2E9 cells    | 50 mL                  | 2 mg   | 2 mL                 |
|                        | 100 L          | 2E11 cells   | 5 L                    | 200 mg | 200 mL               |
| 2.5E6 cells/mL         | 30 mL          | 7.5E7 cells  | 1.5 mL                 | 75 µg  | 75 µL                |
|                        | 1 L            | 2.5E9 cells  | 50 mL                  | 2.5 mg | 2.5 mL               |
|                        | 100 L          | 2.5E11 cells | 5 L                    | 250 mg | 250 mL               |

## Notes

1. To improve transfection efficiency, it is recommended to adapt suspended cells in a serum-free culture system for a few days before transfection.
2. High-quality plasmids are recommended for transfection, such as plasmids free of endotoxins, proteins, and RNA residues.
3. For your safety and health, please wear a lab coat and disposable gloves, and operate in a fume hood.