A General Protocol for PEIone™ Transfection Reagent

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This Product is for Research Use Only -

Not for Diagnostic or Treatment Purposes

SKU: AR000000

Product Description

PEIone™ is a linear polyethylenimine (PEI)-based transfection reagent specifically optimized for high-efficiency adeno-associated virus (AAV) production in suspension and adherent HEK 293T or HEK 293 cell systems. As a chemically synthesized, animal-origin–free reagent, PEIone™ ensures low cytotoxicity while maintaining robust and reproducible transfection performance.

Supplied as a clear, sterile, ready-to-use liquid, PEIone™ is fully compatible with our novel AAVone® single-plasmid system, and other plasmid-based AAV production systems, such as our AAVdual® two-plasmid system and traditional AAVtri three-plasmid system, making it broadly applicable in both research and large-scale AAV manufacturing.

Storage

• Store at 2–8 °C • Stable for up to 2 years • Do not freeze

Key Advantages

• High DNA-binding capacity with efficient performance at low reagent-to-DNA ratios
• Uses only one-third of the reagent, reducing consumption and overall cost
• Stable complex formation ensuring consistent transfection efficiency
• Scalable for both research- and industrial-scale AAV production

Protocol

1. Required Materials

• HEK 293 or HEK 293T cells (suspension or adherent).
• Plasmid DNA (pAAVone® plasmids or other plasmids for AAV production; 0.5–5 μg/μL stock).
• Appropriate cell culture medium.

2. Preparing Cells

• Suspension cells: Grow cells to a density of 4.0–6.0 × 10^6 viable cells/mL with ≥95% viability. Dilute to 2.5–3.5 × 10^6 viable cells/mL using pre-warmed medium at the time of transfection.
• Adherent cells: Seed cells to reach 70–90% confluence at the time of transfection.

3. Transfection Protocol for 240 ml culture

• Bring PEIone™ and culture medium to room temperature.
• Dilute plasmid DNA in culture medium to ~5% of the culture volume (e.g., 360 μg DNA in 12 mL medium for 240 mL culture). Mix gently.
• In a separate tube, dilute PEIone™ reagent in culture medium to ~5% of the culture volume (e.g., 126 μL PEIone™ reagent in 12 mL medium for 240 mL culture). Mix gently.
• Add diluted PEIone™ solution to the diluted DNA solution. Mix gently.
• Incubate for 15–30 minutes at room temperature to allow complex formation.
• Add complexes directly to the culture vessel.
• Incubate cells for about 68-72 hours, then harvest according to in-house AAV production protocols.

Recommended DNA and Reagent Amounts

The amount of PEIone™ and DNA, and their ratios should be optimized for your AAV genomes and serotypes/capsids to obtain maximum productivity.

Typical PEIone™/ pAAVone® Ratios:

• Suspension HEK 293 or HEK 293T cells: PEIone™/DNA = 0.5:1–1:1
• Adherent HEK 293 or HEK 293T cells: PEIone™/DNA = 1:1–2:1

Typical pAAVone® amount:

• Suspension HEK 293 or HEK 293T cells: 0.3-0.6 µg per 1 × 10^6 cells
• Adherent HEK 293 or HEK 293T cells: 0.3-0.6 µg per well (24-well plate)

Typical PEIone™ amount:

• Suspension HEK 293 or HEK 293T cells: 0.2-0.5 µl per 1 × 10^6 cells
• Adherent HEK 293 or HEK 293T cells: 0.5-1.0 µl per well (24-well plate)

Table 1. Recommended DNA amounts and reagent volumes for transfection of pAAVone® plasmid at various scales in suspension HEK 293/293T cells

	Vessel type				
	6-well	125 mL flask	250 mL flask	500 mL flask	1 L flask
Cell number	6 × 10^6	90 × 10^6	180 × 10^6	360 × 10^6	720 × 10^6
Culture volume	2 mL	30 mL	60 mL	120 mL	240 mL
DNA amount	3 µg	45 µg	90 µg	180 µg	360 µg
PEIone™	2.1 µL	31.5 µL	63 µL	126 µL	252 µL
Dilution medium	2 × 100 µL	2 × 1.5 mL	2 × 3 mL	2 × 6 mL	2 × 12 mL

Table 2. Recommended DNA amounts and reagent volumes for transfection of pAAVone® plasmid at various scales in adherent HEK 293/293T cells.

	Vessel type					
	24-well	12-well	6-well	T-25 flask	T-75 flask	100 mm dish
Culture medium	0.5 mL	1 mL	2 mL	4 mL	10 mL	10 mL
DNA amount	0.5 µg	1 µg	2 µg	4 µg	8 µg	8 µg
PEIone™	0.75 µL	1.5 µL	3 µL	6 µL	12 µL	12 µL
Dilution medium	2 × 25 µL	2 × 50 µL	2 × 100 µL	2 × 200 µL	2 × 0.5 mL	2 × 0.5 mL

Notes

For triple-plasmid AAV systems (AAVtri), the total plasmid amount required is typically higher than that for pAAVone®. The ratio of the three plasmids should be optimized for maximum AAV productivity. A starting molar ratio of 1:1:1 for each plasmid is recommended for initial testing.