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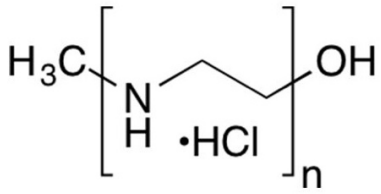
Transfection Grade PEI (MW 40,000)

Catalog Number	Packaging Size
P017-1	100 mg
P017-2	1 g

Storage upon receipt: 2-8°C

The transfection grade polyethylenimine (PEI) is a powerful, trusted, and cost-effective reagent widely considered as a current gold standard for both *in vitro* and *in vivo* transfection. PEI has a high density of protonatable amino groups, with amino nitrogen as every third atom. This imparts a high buffering ability at nearly any pH. Hence, once inside the endosome, PEI disrupts the vacuole and releases the genetic material into the cytoplasm. Stable complexation with DNA, efficient entry into the cell, and ability to escape the endosome makes PEI a highly efficient transfection reagent which is compatible for a wide range of cell lines/types including the most commonly used HEK293 and CHO cells grown in adherent and suspension cultures.

For research use only.



Technical Information

Appearance:	White solid
CAS Number:	49553-93-7
Molecular Formula:	$(CH_2CH_2NH•HCl)_n$
Molecular Weight:	average M_n 40000
PDI:	<1.3
Storage Condition:	2-8°C
Shipping Condition:	Room temperature

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Liquid Formulation Protocol

Procedure:

1. Accurately weigh and dissolve 100 mg of PEI powder in 80 mL of ddH_2O. Mix for several minutes to dissolve powder.

2. Gradually add 1 M NaOH to PEI solution while monitoring with pH meter to adjust pH to 7.0 ± 0.1.

3. Add ddH_2O to bring PEI solution to bring total volume to 100 mL.

4. Sterile-filter the solution with 0.2 µm syringe filter into sterile storage container.

5. Label and store container at 4°C for up to 12 months. - Do not freeze.

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Protocol for

Transient Gene Expression in CHO Suspensions

General Guidance

PEI transfection is inhibited by serum. Use media that is reduced-serum, serum-free ("SFM") or chemically defined ("CDM").

Cell densities used are for typical CHO cultures with maximum viable cell densities of ~4.0×10^6 cells/mL. If using a high-density system, increase the values for cell density linearly. For example, ExpiCHO™ media and ExpiCHO-S™ cells can support viable cell densities over ~12 ×10^6 cells/mL. Accordingly, this system should be transfected at 3.0×10^6 cells/mL instead of 1.0×10^6 cells/mL.

The typical pDNA and PEI concentrations (1.0 and 5.0 mg/L, respectively) can achieve high transfection efficiency at viable cell densities from 1.0×10^6 cells/mL up to 5.0×10^6 cells/mL.

If performing the same expression many times, or many similar expressions, we would recommend co-varying on these parameters over corresponding ranges to find the optimal conditions:

Parameter	Range
PEI Concentration	3.50-6.50 mg/(L final culture)
DNA Concentration	0.75-1.50 mg/(L final culture)
PEI/DNA Complex Time	5.0 to 15.0 minutes

Before Transfection

Subculture and expand cells to obtain culture with viability greater than 95% and viable cell density between 1.5-2.0 ×10^6 cells/mL at time of transfection.

Transfection

1. Immediately prior to transfection, ensure that viability is greater than 95% and viable cell density is 1.5-2.0 ×10^6 cells/mL.

2. Dilute the viable cell density to 1.0 ×10^6 cells per mL with media.

3. Invert pDNA and PEI 1 mg/mL reagent containers several times to mix well.

4. To a clean vial, transfer 1 µg pDNA for each mL of culture to be transfected. Dilute to 20 µg/mL with fresh media.

5. To a clean vial, transfer 5 µL PEI 1 mg/mL for each mL of culture to be transfected. Use media to dilute to 75 µg/mL.

6. Mix together diluted pDNA and PEI. Invert several times and allow to rest capped at room temperature for 10 minutes. Gently invert the closed container one time immediately before use.

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7. Use 10 mL of pDNA/PEI mixture for each 90 mL of culture to be transfected. Gradually add mixture to culture while mixing.

8. Incubate cells per typical conditions.

Post Transfection

If using a feed, booster, supplement, or enhancer, these can be added any time six hours post-transfection. Subcultures can also be prepared after six hours.

Depending on the cell culture media, it may be necessary to add sodium butyrate to the media to obtain gene expression in CHO suspensions. To determine if necessary, prepare 5 post-transfection subcultures and add sodium butyrate to final concentrations of 0, 2.5, 5, 7.5, or 10 mM to find appropriate concentration based on the final yields. Sodium valproate can be used instead.

If using a GFP control, transfection efficiencies over 70% should be observed after 48 hours. For optimized procedures, efficiencies over 80% are reasonable.

Monitor expression levels and harvest upon observing plateaued titers. For typical processes, secreted proteins will be highest at 5 to 7 days post transfection. Other processes, such as using low temperatures and/or using a batch feed to obtain maximal yields, will change the peak harvest window.

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Protocol for

Transient Gene Expression in HEK293 Suspensions

General Guidance

PEI transfection is inhibited by serum. Use media that is reduced-serum, serum-free ("SFM") or chemically defined ("CDM").

Cell densities used are for typical HEK293 cultures with maximum viable cell densities of ~4.0×10^6 cells/mL. If using a high-density system, increase the values for cell density linearly. For example, Expi293™ media and Expi293F™ cells can support viable cell densities over ~12 ×10^6 cells/mL. Accordingly, this system should be transfected at 3.0×10^6 cells/mL instead of 1.0×10^6 cells/mL.

The typical pDNA and PEI concentrations (1.0 and 3.0 mg/L, respectively) can achieve high transfection efficiency at viable cell densities from 1.0×10^6 cells/mL up to 5.0×10^6 cells/mL.

If performing the same expression many times, or many similar expressions, we would recommend co-varying on these parameters over corresponding ranges to find the optimal conditions:

Parameter	Range
PEI Concentration	1.50-4.50 mg/(L final culture)
DNA Concentration	0.75-1.50 mg/(L final culture)
PEI/DNA Complex Time	5.0 to 15.0 minutes

Before Transfection

Subculture and expand cells to obtain culture with viability greater than 95% and viable cell density between 1.5-2.0 ×10^6 cells/mL at time of transfection.

Transfection

1. Immediately prior to transfection, ensure that viability is greater than 95% and viable cell density is 1.5-2.0 ×10^6 cells/mL.

2. Dilute the viable cell density to 1.0 ×10^6 cells per mL with media.

3. Invert pDNA and PEI 1 mg/mL reagent containers several times to mix well.

4. To a clean vial, transfer 1 µg pDNA for each mL of culture to be transfected. Dilute to 20 µg/mL with fresh media.

5. To a clean vial, transfer 3 µL PEI 1 mg/mL for each mL of culture to be transfected. Use media to dilute to 60 µg/mL.

6. Mix together diluted pDNA and PEI. Invert several times and allow to rest capped at room temperature for 10 minutes. Gently invert the closed container one time immediately before use.

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7. Use 10 mL of pDNA/PEI mixture for each 90 mL of culture to be transfected. Gradually add mixture to culture while mixing.

8. Incubate cells per typical conditions.

Post Transfection

If using a feed, booster, supplement, or enhancer, these can be added any time six hours post-transfection. Subcultures can also be prepared after six hours.

If using a GFP control, transfection efficiencies over 70% should be observed after 48 hours. For optimized procedures, efficiencies over 80% are reasonable.

Monitor expression levels and harvest upon observing plateaued titers. For typical processes, secreted proteins will be highest at 5 to 7 days post transfection.

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Protocol for

AAV Production in HEK293 Suspensions

General Guidance

This procedure describes a general method to use PEI to prepare rAAVs in a 50 mL suspension of HEK293 cells. The methods can be scaled out to prepare multiple rAAVs or scaled up to prepare greater quantities of a desired rAAV. Compared with transection of adherent 293 cells, transfection of suspension 293 cells is easier to scale up.

Procedure

1. Four days prior to the transfection, count viable cell density of a cell culture and use fresh media to dilute to 0.6 $\times 10^6$ viable cells/mL.
2. On the day of transfection, dilute and aliquot the cell culture to obtain 45 mL of a subculture with a viable cell density of 1.1 $\times 10^6$ cells/mL.
3. Prepare a pDNA mixture in a vial by combining equimolar amounts of all plasmids to be co-transfected in fresh media to obtain 2.5 mL of a mixture with a total pDNA concentration of 20 µg/mL.
4. Prepare a PEI mixture in a vial by diluting 125 µL of PEI solution (1.0 mg/mL) with fresh media to obtain 2.5 mL of a mixture with a PEI concentration of 50 µg/mL.
5. Prepare the transfection mixture by combining the pDNA and PEI mixtures and briefly vortex for a few seconds. Allow the transfection mixture to rest for 5-10 minutes before use. Do not use transfection mixture more than 15 minutes after mixing.
6. Transfect the 45 mL cell culture by slowly adding the 5 mL transfection mixture while swirling. Return the cell culture to incubation.
7. rAAVs can be harvested and purified 48-96 hours post-transfection.

Frequently Asked Questions (FAQs)

Q1. How should this procedure be modified if all the AAV genes are on one plasmid instead of two plasmids?

A1. The plasmid containing the AAV genes should be mixed with the transgene cassette plasmid in equimolar amounts to obtain a pDNA mixture with a concentration of 20 µg/mL, and a final pDNA concentration of 1 µg/mL in the cell culture.

Q2. Should I increase pDNA and PEI concentrations when using a high-density cell culture?

A2. No. If using a high-density cell culture, the transfection should be performed at 3.3 $\times 10^6$ cells /mL instead of 1.1 $\times 10^6$ cells/mL, but the final pDNA concentration should still be 1 µg/mL and the final PEI concentration should still be 2.5 µg/mL.

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Protocol for

Transient Gene Expression in Adherent HEK293 Cells

General Guidance

PEI transfection is inhibited by serum. Use media that is reduced-serum, serum-free ("SFM") or chemically defined ("CDM").

We list recommended reagent quantities per 10^6 cells in the table below. If performing many very similar transfections, we would recommend co-varying on these parameters over optimization ranges first.

Parameter	Recommended	Optimization Range
PEI Concentration (per 10^6 cells)	3.0 µg	2.0-4.0 µg
DNA Concentration (per 10^6 cells)	1.5 µg	1.0-2.0 µg
PEI/DNA Complex Time	10 minutes	5.0 to 15.0 minutes

Even if performing co-transfection , keep the total DNA concentration range the same (1.0 to 2.0 µg/10^6 cells).

If high toxicity is observed, then reduce the quantity of PEI and/or DNA first. We do not recommend replacing the media post-transfection to reduce toxicity except as a last resort.

Before Transfection

The day before transfection, seed the cells the day before transfection to reach 50-80% confluence on the day of transfection. Seed approximately 50,000 cells per cm^2.

Transfection

1. Measure the cell density to determine the transfection parameters based on the number of viable cells.
2. Dilute 1.5 µg pDNA per 10^6 cells in 5% final culture volume of DMEM or media. Mix well.
3. Dilute 3.0 µg PEI per 10^6 cells in 5% final culture volume of DMEM or media. Mix well.
4. Mix the solution of pDNA and PEI together by quickly and briefly vortexing or inverting the tube several times.
5. Allow the mixture of pDNA and PEI to rest for 10 minutes at room temperature.
6. Gradually add the pDNA and PEI mixture to the cells dropwise while swirling the plate.
7. Incubate the cells at typical incubation conditions.

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Protocol for

AAV Production in Adherent HEK293 Cells

General Guidance

This procedure describes a general method to use PEI to prepare rAAVs in a 6-well plate. The method can be scaled out to prepare multiple rAAVs and/or scaled up to prepare greater quantities of a desired rAAV. While transfection of a 293 suspension is recommended, transfection of adherent 293 cells may be faster and more convenient in some situations.

Procedure

1. The day before transfection, trypsinize and count the cells. Plate 3×10^5 cells in 2.0 mL of complete growth medium, then incubate. On the day of transfection, cells should be 50% to 80% confluent.
2. Prepare a pDNA mixture in a vial by combining equimolar amounts of all plasmids to be co-transfected, total 2.5 µg, diluted in fresh media to 250 µL.
3. Prepare a PEI mixture in a vial by diluting 6 µL of PEI solution (1.0 mg/mL) with fresh media to obtain 250 µL of a mixture.
4. Prepare the transfection mixture by combining the pDNA and PEI mixtures and mix by pipetting for a few seconds. Allow the transfection mixture to rest for 5-10 minutes before use. Do not use transfection mixture more than 15 minutes after mixing.
5. Transfect the cell culture by slowly adding the 500 µL transfection mixture to the well while swirling. Return the cell culture to incubation.
6. rAAVs can be harvested and purified 48-96 hours post-transfection, with the appropriate harvest methods depending on the AAV serotype.

Frequently Asked Questions (FAQs)

Q1. How should this procedure be modified if all the AAV genes are on one plasmid instead of two plasmids?

A1. The plasmid containing the AAV genes should be mixed with the transgene cassette plasmid in equimolar amounts to obtain a pDNA mixture with a concentration of 20 µg/mL, and a final pDNA concentration of 1 µg/mL in the cell culture.

Q2. How should I change this protocol for different cell culture vessels?

A2. The following table details recommended parameters for adherent 293 transfection:

Culture Vessel	Plating Medium Volume	Cells per well	Total DNA	Total PEI	Total Dilution Volume
96-well	100 µl	2.5×10^4	125 ng	300 ng	20 µl
24-well	500 µl	1.25×10^5	0.6 µg	1.5 µg	100 µl
12-well	1 ml	2.5×10^5	1.25 µg	3.0 µg	200 µl
6-well	2 ml	5.0×10^5	2.5 µg	6.0 µg	500 µl
6-cm dish	5 ml	1.25×10^6	6.25 µg	15.0 µg	1 ml
10-cm dish	15 ml	3.75×10^6	18.5 µg	45.0 µg	3 ml

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Protocol for

Lentivirus Production in Adherent HEK293 Cells

General Guidance

This procedure describes a general method to use PEI to prepare lentiviruses in a 10 cm dish of HEK293T cells, which can be scaled up to larger cell cultures to prepare greater numbers of lentiviruses or scaled out to prepare many different lentiviruses.

Procedure

1. The day before transfection, count and split cells to seed ~2.5×10^6 HEK293T cells in 10 mL fresh medium. Return dish to incubator.
2. On the morning of the transfection, check that the confluence is between 60% and 70%, replace medium with 10 mL fresh medium and return the dish to the incubator.
3. In a 1 mL vial, prepare a pDNA mixture of 10 µg pDNA in 500 µL fresh medium that contains equimolar quantities of the three lentiviral packaging and envelope gene plasmids, along with two molar equivalents of the lentiviral transgene plasmid.
4. In a separate 1 mL vial, prepare a PEI mixture that contains 25 µg PEI in 500 uL fresh medium.
5. Combine the pDNA mixture and PEI mixture to create a transfection mixture by pipetting, using the pipet the gently mix. Allow the transfection mixture to rest without further agitation for 5-10 minutes. Do not use if the transfection mixture rests for more than 15 minutes.
6. Gently add the transfection mixture dropwise to the cell culture while swirling. Return the dish to the incubator.
7. Approximately 24 hours after transfection, replace the transfection medium with 10 mL fresh medium, along with any desired booster supplements (e.g., sodium butyrate, Trichostatin A, etc.). Store spent medium in a 50 mL vial at 4°C. Return dish to incubator.
8. At approximately 48 hours after transfection, replace the transfection medium with 10 mL fresh medium, along with any desired booster supplements (e.g., sodium butyrate, Trichostatin A, etc.). Collect spent medium in the same 50 mL vial used to collect the supernatant at 24 hours, and store at 4°C. Return dish to incubator.
9. At approximately 72 hours after transfection, collect the supernatant in the same 50 mL vial used in the previous steps and store at 4°C. The cells can be disposed of.
10. The viruses in the supernatant can be tested, purified, and concentrated as needed. Aliquots of lentiviruses can be stored at -80°C indefinitely.

Frequently Asked Questions (FAQs)

Q1. Can I use this protocol for second-generation lentivirus production?

A1. Yes. The copies of the envelope, packaging, and transfer plasmids should be increased to maintain the 10-µg total pDNA used for transfection in a 10 cm dish.