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Transfection for Recombinant Antibodies

Addgene The Nonprofit Plasmid Repository[1]

[1]Addgene

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DISCLAIMER

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ABSTRACT

This protocol describes how to transfect suspension HEK293 cells with recombinant antibody plasmids using Polyethylenimine Max as a transfection reagent. After transfection and expression, the recombinant antibody can be purified for use in a variety of applications.
See the original protocol on Addgene's website:
https://www.addgene.org/protocols/transfection-for-recombinant-antibodies/

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EXTERNAL LINK

https://www.addgene.org/protocols/transfection-for-recombinant-antibodies/

PROTOCOL CITATION

Addgene The Nonprofit Plasmid Repository 2022. Transfection for Recombinant Antibodies. **protocols.io**
https://protocols.io/view/transfection-for-recombinant-antibodies-b6qfrdtn

KEYWORDS

recombinant antibodies, transfection, HEK293, Polyethylenimine Max (PEI-MAX)

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GUIDELINES

General Considerations:
For antibody-expressing plasmids containing the SV40 origin of replication use a HEK293 line stably expressing the SV40 large T antigen.

For antibody-expressing plasmids containing the Epstein-Barr virus origin of replication use a HEK293 line stable expressing Epstein Barr nuclear antigen (EBNA).

Workflow Timeline
Day 1: Seed cells
Day 2: Transfect cells
Day 3-6: Feed cells
Day 7: Harvest antibody

Tips and Troubleshooting:
We recommend wiping down all pipettes and equipment with 10% bleach prior to use.

MATERIALS TEXT

Equipment:

- Class II, Type A2 Biological Safety Cabinet (Tissue Culture hood)
- 4 °C Refrigerator
- Benchtop centrifuge compatible with 50 mL conical tubes
- 37 °C, 5% CO_2 incubator with shaking platform set to 120 rpm
- 37 °C bead bath
- Automated cell counter
- Pipet controller
- 0.5-10 µL single channel pipettor
- 2-20 µL single channel pipettor
- 20-200 µL single channel pipettor
- 0.1-1 mL single channel pipettor
- Vortex

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- Stir bar
- Magnetic stir plate
- pH meter

Reagents and Consumables:

- White disposable sleeves, DuPont IC501BWH000100CS
- Kimwipes, VWR 82003-822
- Microcentrifuge tubes, VWR 89126-724
- 50 mL conical tubes, VWR 89039-656
- 500 mL vented flask, 431147
- Pipettes and micropipette tips
- Cell counting chamber slide, Thermo Fisher C10228
- 30 mL Luer-lock syringe, BD BD302832
- 0.2 µm Luer-lock filter, VWR 431229
- 0.22 µm PES filtering system, 1000 mL, VWR 431098
- 0.45 µm PES filtering system, 500 mL, VWR 430770
- Trypan Blue, Thermo Fisher T10282
- 10% Pluronic F-18, Thermo Fisher 24040032
- Polyethylenimine hydrochloride, M.W. 40000 (PEI-MAX), Linear, Transfection Grade, VWR 75800-188
- Valproic acid sodium salt, Sigma Aldrich P4543-10G
- Glutagro, Corning 25-015-CI
- BalanCD HEK293 Media, Irvine Scientific 91165-1L
- BalanCD HEK293 Feed, Irvine Scientific 91166-500ML
- Benzamide, Millipore Sigma 12072
- Antipain, Millipore Sigma 10791
- Leupeptin, Millipore Sigma L2884
- Aprotinin saline solution, Millipore Sigma A6279
- HEK293 cells
- Recombinant antibody plasmid DNA

Reagent Preparation:

100 mM Valproic Acid

- Dissolve **2.88 g** valproic acid in **200 mL** deionized water.
- Sterilize by passage through a sterile 0.22 µm PES filter.
- Prepare 10 mL aliquots and store the solution at **-20 °C** .

1 mg/mL PEI-MAX

- Add **1 g** of PEI-MAX powder to **900 mL** deionized water in a 1 L bottle.
- Add stir bar and stir on a magnetic stir plate.

The powder should dissolve rapidly (with 20 min) but check for the presence of particles still in solution. Continue to stir until all particles have dissolved. This may take several hours.

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- Adjust to pH 7.0 as needed with 10 N sodium hydroxide (NaOH) or 5 N hydrochloric acid (HCl).

> Adjust the pH slowly, adding no more than 200 µL of NaOH or 20 µL of HCl at a time.

- Add deionized water to a final volume of 1 L . Check to ensure the pH has not drifted.
- Sterilize the solution by passing the solution through a 0.22 µm PES filter apparatus.
- Prepare aliquots and store at -20 °C until use.

BCD TFX

- 1000 mL BalanCD HEK293 Media
- 10 mL 10% Pluronic-F68
- 40 mL 200 mM Glutagro
- Do not add selective reagents.
- Store at 4 °C until use. We suggest preparing fresh solutions after one month.

BCD Feed

- 500 mL BalanCD HEK293 Feed
- 20 mL 200 mM Glutagro
- Store at 4 °C until use. We suggest preparing fresh solutions after one month.

1000X protease inhibitor cocktail

- 25 mg leupeptin
- 50 mg antipain
- 250 mg benzamidine
- 25 mL of 2 mg/mL aprotinin
- Mix well and sterilize through a 0.2 µm PES filter.
- Aliquot and freeze upright at -20 °C until use.

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SAFETY WARNINGS

HEK293 cells are considered biosafety level 2. Please ensure that you are in compliance with your institution's biosafety regulations.

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BEFORE STARTING

See the Materials section for preparation of necessary stock solutions.

Warm the DNA and working stock of PEI-MAX to room temperature before use, and warm the BalanCD HEK293 transfection media (BCD TFX) to 37 °C before use.

Seeding cells

1 One day prior to transfecting, seed a **108 mL** of culture of cells at a density of **0.9*10^6 cells/mL** in a 500 mL vented flask.

> Do not use cells that are over 30 passages.

2 Check cell density and viability:

2.1 Transfer the flask of HEK cells in culture into the biosafety cabinet (BSC).

> Do not get the filter cap wet.

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2.2 Transfer **10 µL** of trypan blue into a clean microcentrifuge tube.

2.3 Vortex the cell suspension.

2.4 Transfer **10 µL** of cell suspension into the microfuge tube containing the trypan blue.

2.5 Pipette 10 times to mix.

2.6 Load **10 µL** of the cell suspension/trypan blue mix into one chamber of a cell counting chamber.

2.7 Load the cell counting chamber on an automated cell counter and measure the live cell density and viability of the culture.

2.8 Determine the volume of cell suspension required to seed **108 mL** of media at a final density of **0.9*10^6 cells/mL** .

Refer to the following example for calculating the dilution:
If live cell density = $3 * 10^6$ cells/mL:

$$C1 * V1 = C2 * V2$$

$$3 * 10^6 \text{ cells/mL} * V1 = (0.9 * 10^6 \text{ cells/mL}) * 108 \text{ mL}$$

$$V1 = 32.4 \text{ mL}$$

You would dilute 32.4 mL of the initial $3 * 10^6$ cells/mL culture to a final volume of 108 mL in a 500 mL vented flask.

3 Transfer **108 mL** of BCD TFX media into each of a 500 mL vented flask.

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4 Cap the flask and equilibrate media in a **37 °C** , 5% CO_2 incubator for **01:00:00** . 1h

5 Transfer the required volume of cells to seed each flask with **108 mL** at **0.9*10^6 cells/mL** to centrifuge bottles.

6 Centrifuge to pellet cells, **100 x g, 00:10:00** 10m

7 Aspirate supernatant.

8 Resuspend pellets in **10 mL** of the pre-equilibrated media and transfer back into the flask, with total volume **108 mL** .

9 Cap the flask.

10 Incubate in a **37 °C** , 5% CO_2 incubator on a shaking platform set to **120 rpm** .

Transfection

11 Place the flask containing HEK293 cells seeded the previous afternoon in the BSC.

Do not get the filter cap wet.

12 Using a 5 mL pipette, transfer **0.5 mL** of cell suspension into a clean microcentrifuge tube.

Cells settle quickly and need to be resuspended before sampling. Gently swirl the flask 5-

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10 times before sampling.

13 Transfer **10 µL** of trypan blue into a clean microcentrifuge tube.

14 Vortex the cell suspension.

15 Transfer **10 µL** of cell suspension into the microfuge tube containing the trypan blue.

16 Pipette 10 times to mix.

17 Load **10 µL** of the cell suspension/trypan blue mix into one chamber of a cell counting chamber slide.

18 Load the cell counting chamber on an automated cell counter and measure the live cell density and viability of the culture.

Culture should be between 1.5 - 2 * 10^6 cells/mL with >95% viability to proceed with transfection.

19 Transfect the flask containing **108 mL** cells as follows:

19.1 Transfer **6 mL** of BCD TFX into each of **two** 50 mL tubes.

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19.2 Cap the tubes and incubate for **01:00:00** in the **37 °C** bead bath to warm. 1h

19.3 Transfer the PEI-MAX and recombinant antibody plasmid DNA sample to the BSC and incubate for **01:00:00** at **Room temperature** . 1h

19.4 After the BCD TFX has warmed in the bead bath, transfer the tubes to the BSC.

19.5 Add **180 µg** of recombinant antibody plasmid DNA to one tube of **6 mL** BCD TFX.

19.6 Cap the tube and vortex for 5 seconds to mix.

19.7 Add **450 µg** of 1 mg/mL PEI-MAX to the second tube of **6 mL** BCD TFX. (For a 1 mg/mL stock solution of PEI-MAX, 450 µg = 450 µL)

> The optimal ratio of DNA:PEI may vary significantly and should be empirically determined for your sample. Typical ratios may range from 1:1 to 1:6.

19.8 Cap the tube and vortex for 5 seconds to mix.

19.9 Add the diluted PEI-MAX to the diluted DNA.

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19.10 Cap the tube and vortex with three 1-second pulses.

19.11 Incubate for **00:03:00** at **Room temperature** . 3m

19.12 Transfer the 500 mL flask of HEK293 cells to the BSC.

19.13 Add **12 mL** of transfection mix to the flask dropwise.

19.14 Cap the flasks and swirl 5-10 times to mix.

20 Return the flask to the incubator.
37 °C

BCD feed and valproic acid supplementation 1w

21 During the 24-144 h post transfection, supplement the flask with 4% BCD feed.

The feed can be repeated up to 4 times for a total of 16% of the culture volume.

22 At 72-96 h post transfection, supplement the flask with **3.75 millimolar (mM)** valproic acid.

Example feeding strategy:
Thursday: Transfect cells.
Friday (24 h post transfection): Add 4% BCD feed.
Monday (96 h post transfection): Add valproic acid to 3.75 mM, add 4% BCD Feed.
Tuesday (120 h post transfection): Add 4% BCD feed.

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Wednesday (144 h post transfection): Add 4% BCD feed.
Thursday (168 h post transfection): Harvest antibody

Harvesting antibody 15m

23 At 168 hours (1 week) post-transfection, harvest the antibody:

24 Transfer the HEK293 cells and media to 50 mL conical tubes.

25 Centrifuge to pellet the cells, **3100 x g, 00:15:00** 15m

26 Filter the supernatant through a 0.45 µm PES filter.

27 Add 1X protease inhibitor cocktail to the supernatant.

28 The supernatant containing the recombinant antibody can be used as-is or the recombinant antibody can be purified from the supernatant.

See our Recombinant Antibody Purification Protocol

29 Store the supernatant at **4 °C** until ready to use.

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