Plasmid Transfection Protocol

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A Charles River Company

Before you get started

Do your research

The following protocols are intended to be general guidelines and are not optimized for your specific cell line. We recommend that you do a literature search to find a protocol that closely aligns with your experimental conditions for optimal results.

Use high-quality DNA and healthy cells

It is important to use plasmids that are free of contaminants, such as protein, RNA, or endotoxins that may reduce transfection effeciency. You should also only use cell lines that have been passaged a few times and are in good health.

Transfection

1. Split the cells to achieve ~70% confluency for the day of transfection.

2. One hour prior to transfection, change the cell culture media to a media without FBS (serum-free media).

3. Dilute your DNA in media to the appropriate amount that you will need to use based on the size of your wells.
    a. E.g., For a 24 well plate you can use 2 ug of DNA and 10 uL of polyethylenimine (PEI) at 1 mg/mL per well. You can also alter the ratio of DNA:PEI from 1:1 to 1:6 to optimize your transfection efficiency.
    b. Add PEI mixture to the DNA mixture and vortex.

4. Incubate the DNA-PEI master mix for 20 minutes at room temperature

5. Add DNA-PEI master mix into each well and place cells back into the incubator.

6. Remove the serum-free media and replace with serum-containing media overnight.

7. Harvest cells 1-6 days post-transfection.

Note: This protocol is written for transfecting HEK293T cells and may not be optimal for your cell line.