Enhanced Transfection of Mammalian Cells with Polybrene

This is intended as a guide only; please consult the references and wider literature for more details.

In Brief

Step 1: Prepare DNA Plasmid

Sufficient plasmid DNA must first be generated. Begin by amplifying the quantity of the plasmid by performing bacterial transformation. Grow the transformed bacterial culture overnight (in 37°C shaker) and extract the plasmid DNA using DNA MAXIprep kit.

Step 2: Prepare HEK Cells

Culture HEK293T cells in HEK media (DMEM/F-12 media, FBS, non-essential amino acids, L-glutamine) within humidified incubators (standard conditions of 37˚C, 5% CO_2 and 21% O_2). Once the culture has reached high confluency (>90%), the cells are ready for transfection.

Step 3: Prepare Transfection Media

- Immediately before transfection, replace the HEK culture media with DMEM.
- Prepare the transfection media, which consists of an optimized ratio of DMEM, DNA plasmid, lipofection reagent and polybrene stock solution (see Table 1). Typically, polybrene is used at a concentration of 4 µg/mL - 10 µg/mL (see Table 2).

Step 4: Transfection

- Gently mix the transfection media before adding dropwise to the culture plate, to prevent damaging the cells.
- Place the cells immediately in the incubator with the following settings overnight: 35˚C, 3% CO_2 and 21% O_2.

Step 5: Harvesting

- Return culture to standard maintenance incubator. Leave the cells for 48-72 h post-transfection before proceeding to downstream application (i.e., harvesting of virus from media).

Table 1.

Polybrene (cat. No. 7711) is supplied as a 10 mg solid and is soluble in water (up to 100 mg/mL). To generate a working solution, Polybrene should first be dissolved in 1 mL sterile water to generate a 10 mg/mL stock solution.

Stock solution Polybrene concentration (mg/mL)	Stock volume (mL)	Stock Polybrene (Solid, mg)	Sterile Ultra-Pure Water (mL)
10	1	10	1

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Table 2.

Polybrene working solution is generated from the polybrene stock solution (10 mg/mL in sterile water). This stock solution can be further diluted in the culture media to generate the desired working concentration for the transfection or transduction media. Example dilutions shown below.

Final desired working concentration (µg/mL)	Dilution	Stock solution Polybrene concentration (mg/mL)	Volume of stock solution Polybrene (µL)	Volume of culture media (mL)
4	1:2500	10	20	50
6	3:5000	10	30	50
8	1:1250	10	40	50
10	1:1000	10	50	50

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