

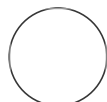


APR 15, 2023

Generation of stable cell lines using retroviral system

nguyen.tha¹

¹Laboratory of Michael Lazarou, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia



nguyen.tha

ABSTRACT

This protocol details generation of stable cell lines using retroviral system.

ATTACHMENTS

[698-1486.docx](#)

GUIDELINES

Attention

- The HEK293T cells detach very easily, be extra gentle when changing the media.

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.81wgbyez1vpk/v1

Protocol Citation: nguyen.tha 2023. Generation of stable cell lines using retroviral system. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.81wgbyez1vpk/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Apr 10, 2023

Last Modified: Apr 15, 2023

PROTOCOL integer ID:
80233

Keywords: stable cell lines, retroviral system

MATERIALS

Buffers and reagents:

- Polybrene (4 mg/mL)

Growth media:

A	B
DMEM with 10% FBS	
Glucose	4.5 g/l
GlutaMAX™	1x
MEM NEAA	1x
HEPES	25 mM

⊗ 45% D-(-)-Glucose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #G8769**

⊗ GlutaMAX™ Supplement **Thermo Fisher Catalog #35050061**

⊗ MEM Non-Essential Amino Acids Solution (100X) **Thermo Fisher Scientific Catalog #11140050**

⊗ HEPES Buffer 1M Solution Cell Culture Grade MP Biomedicals **Fisher Scientific Catalog #ICN1688449**

⊗ Lipofectamine™ LTX Reagent with PLUS™ Reagent **Thermo Fisher Catalog #A12621**

⊗ Gibco™ Opti-MEM™ I Reduced Serum Medium no phenol red **Fisher Scientific Catalog #11-058-021**

⊗ Millex-HV Syringe Filter Unit 0.45 µm PVDF 33 mm gamma-sterilizable sterilized **Merck MilliporeSigma (Sigma-Aldrich) Catalog #SLHVM33RS**

SAFETY WARNINGS



Attention

- All viral waste must be bleached and left under UV light for at least 30' after viral work in TC hoods before disposal.

Day 1

- 1 Seed NIH HEK293T cells into a 6-well plate (900k cells/well if set up in the morning, 950k cells/well if set up in the afternoon).



Note

Set up 1 well for each construct you wish to generate a virus harvest for, can be scaled up according to your need.


Day 2: The following protocol is designed for one well of th...

- 2 Transfect cells with viral and helper vectors using lipofectamine LTX. Combine the following in a 1.5 mL tube:

A	B
viral vector construct (pBMN, pBABE or pMX) containing cDNA of interest	1.5 µg
gag-pol vector	1.0 µg (amount for 1 well)
VSV-G vector	0.5 µg (amount for 1 well)
Opti-MEM (RT)	500 µL



- 3 Add  3 µL of Plus reagent and mix well. Incubate at  Room temperature for

5m

 00:05:00



4


Add  9 μL of Lipofectamin LTX (1:3 ratio of Plus:LTX is standard in the lab but can be adjusted for your own protocol) and vortex for  00:00:15 . Incubate at

20m 15s



 Room temperature for  00:20:00 .

5

Once the 20 min incubation starts, replace the media in each well with  1 mL DMEM/10% FBS media.

6


When the 20 min incubation finishes, add the optimum/liposome mix to the well.

Note

Do it gently on the side of the well.

Day 3

7

In the morning, remove the old media from the HEK293T cells which may contain viruses at this stage) into a beaker of beach and add  1 mL of fresh growth media. The next day, viruses can be harvested for infection.





8

Seed the target cells (about 100k-120k cells) into a 6-well plate if intending to do infection with fresh viruses.


Day 4

9




In the late afternoon, collect viral supernatant from HEK293Ts, spin down at max speed for  00:05:00 to pellet debris and filter through 0.45 μm syringe filters. Viral particles can freshly be used for infection on the cells plated out on day 3 (see below) or can be frozen at  -80 $^{\circ}\text{C}$ for future use.

5m

10

For second harvest, add  1.5 mL fresh growth media back to HEK293T cells for 2 days and harvest again (on Day 6).



- 11 For infection, harvested viruses are topped up with fresh growth media to make up a total of  2 mL .
- 12 Aspirate the media from the target cells.
- 13 Add the  2 mL of virus-containing media (from step 3) to the target cells. Add polybrene to a final concentration of  8 µg/mL to the well and mix well.



Days 5 and 6

- 14 The viruses can be removed from the cells into a beaker of bleach after 24 h (Day 5) or 48 h (Day 6) and fresh media can be added to the wells.
- 15 All waste must be treated as viral waste for at least 3 media changes over 3 days post-infection.