Generation of stable cell lines using retroviral system

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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.

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

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

Created: Apr 10, 2023
MATERIALS

Buffers and reagents:

- Polybrene (4 mg/mL)

Growth media:

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM with 10% FBS</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>4.5 g/l</td>
</tr>
<tr>
<td>GlutaMAXTM</td>
<td>1x</td>
</tr>
<tr>
<td>MEM NEAA</td>
<td>1x</td>
</tr>
<tr>
<td>HEPES</td>
<td>25 mM</td>
</tr>
</tbody>
</table>

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 the 6...

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

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
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<tbody>
<tr>
<td>viral vector construct (pBMN, pBABE or pMX) containing cDNA of interest</td>
<td>1.5 µg</td>
</tr>
<tr>
<td>gag-pol vector</td>
<td>1.0 µg (amount for 1 well)</td>
</tr>
<tr>
<td>VSV-G vector</td>
<td>0.5 µg (amount for 1 well)</td>
</tr>
<tr>
<td>Opti-MEM (RT)</td>
<td>500 µL</td>
</tr>
</tbody>
</table>

3. Add 3 µL of Plus reagent and mix well. Incubate at Room temperature for 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 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 0.45µm 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 °C for future use.

10. For second harvest, add 1.5 mL fresh growth media back to HEK293T cells for 2 days and harvest again (on Day 6).
For infection, harvested viruses are topped up with fresh growth media to make up a total of 2 mL.

Aspirate the media from the target cells.

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

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.

All waste must be treated as viral waste for at least 3 media changes over 3 days post-infection.