Preparing feeder-free hPSCs for nucleofection

In 1 collection

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ABSTRACT

This protocol describes the standard procedure preparing feeder-free human pluripotent stem cells (hPSCs) for nucleofection.

General notes
1. Throughout this protocol, the term hPSC is used to collectively refer to both hiPSCs and hESCs. All described procedures have been tested and work equally well for hiPSCs and hESCs.
2. This protocol is to prepare cells for protocol nucleofection of hPSCs. Before starting, familiarize yourself with the protocol and the required preparations. A detailed protocol on maintaining MEF-cultured hPSCs can be found in the collection "Maintenance and inactivation of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell culture;" doi:
3. Detailed protocols for preparing plasmids, RNA, and RNP for nucleofection can be found in the collection "Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs." A link to this collection can be found in the title section of this protocol, located above.

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PROTOCOL CITATION

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COLLECTIONS

Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs

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Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs

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MATERIALS TEXT

Item	Vendor	Catalog #
DMEM/F12	Thermo Fisher	11320082
DPBS w/o calcium and magnesium	Corning	MT21031CV
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	Thermo Fisher	A14700
Accutase	Thermo Fisher	SCR005
Dispase	STEMCELL Technologies	NC9995391
Countess™ Cell Counting Chamber Slides	Thermo Fisher	C10228

Note: This protocol makes reference to other protocols. Please check for any materials found in those protocols, which might not be listed here

1 Cells are ready for nucleofection when the culture reaches 70-80% confluency

For a detailed protocol on growing feeder-free hPSCs, refer to the collection "Feeder-free culturing of hPSCs;" dx.doi.org/10.17504/protocols.io.b4mcqu2w

2 Coat 6-well plates with VTN/Matrigel/Geltrex as depicted in the collection “Feeder-free culturing of hPSCs," dx.doi.org/10.17504/protocols.io.b4mcqu2w

3 Wash hPSCs with DPBS

4 Use 1 ml Accutase/well of a 6-well plate.

5 Incubate **00:05:00** **37 °C** 5m

6 Add 2 ml DMEM/F12 to each well.

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7 Collect all cells into 15 ml conical tube.

8 Add 7 ml DMEM/F12.

9 Centrifuge at **200-300 x g, 00:05:00** 5m

10 Aspirate supernatant

11 Resuspend cell pellet in 1 ml DMEM/F12, triturate to single cells using P1000 tips

12 Take two sets of 10 µl of cell suspension. Mix each set with 10 µl trypan blue dye, which comes with the Countess™ Cell Counting Chamber Slides

13 Count cells with Countess automated cell counter or hemocytometer, average the counts from the two sets. Continue with re-suspending the cell pellet in 20 ml nucleofection solution as described in the protocol "Nucleofection of hPSCs" (Step 2)

The protocol "Nucleofection of hPSCs" can be found in the collection "Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs." A link to this collection can be found in the title section of this protocol, located above.

14 Mix the cell suspension in the conical tube, take 500,000 cells per nucleofection reaction and transfer to a new conical tube

15 Centrifuge at **200-300 x g, 00:05:00** 5m

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16 Aspirate supernatant

17 Re-suspend cell pellet in 10 ml DPBS

18 Centrifuge at **200-300 x g, 00:05:00** 5m

19 Aspirate supernatant as much as possible, to minimize the interference to the nucleofection buffer system.

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