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Single cell survival assay

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ABSTRACT

This protocol describes the experimental procedure used to measure single cell survival rates post nucleofection of human pluripotent stem cells (hPSCs).

General Notes:

1. Throughout these protocols, 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.

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PROTOCOL CITATION

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MATERIALS TEXT

A	B	C
Item	**Vendor**	**Catalog #**
DMEM/F12	Thermo Fisher	11320082
Fetal Bovine Serum (FBS)	Corning	35-011-CV
Knockout Serum Replacement	Thermo Fisher	10828-028
L-Glutamine	Sigma	G8540
Penicillin & Streptomycin (100X)	Thermo Fisher	15140163
MEM Non-Essential Amino Acids (100X)	Thermo Fisher	11140050
Heat Stable Recombinant Human FGF2	Thermo Fisher	PHG0360
Y-27632	Chemdea	CD0141
2-Mercaptoethanol	Sigma	M3148

1 hPSCs are cultured on MEFs as described in the collection "Thawing, Passaging, and Freezing of hPSCs on MEFS" dx.doi.org/10.17504/protocols.io.b4msqu6e

2 0.5 million hPSCs are nucleofected as described in “Nucleofection of hPSCs" dx.doi.org/10.17504/protocols.io.b4pcqviw

3 Seed 1/100, 1/500 of cells post-nucleofection into one well of a 6-well plate containing hPSCs medium with Rock inhibitor. Prepare at least 2 wells for each seeding density as replicates.

3.1 hPSC medium

Citation: Hanqin Li, Dirk Hockemeyer Single cell survival assay https://dx.doi.org/10.17504/protocols.io.4r3l2okxxv1y/v1

A	B
DMEM/F12	385 ml
Fetal Bovine Serum (FBS)	75 ml
Knockout Serum Replacement	25 ml
L-Glutamine (100X)	5 ml
Penicillin & Streptomycin (100X)	5 ml
MEM Non-Essential Amino Acids (100X)	5 ml
2-Mercaptoethanol (10,000X)	50 µl
Heat Stable Recombinant Human FGF2 (25 µg/ml)*	80 µl

Final volume; 500 mL
*While we prefer Heat Stable Recombinant Human FGF2, we also have used regular FGF2.

L-Glutamine (100x)

A	B
L-Glutamine, powder	14.6 g
MilliQ H2O	500 ml

2-Mercaptoethanol (10,000X)

A	B
2-Mercaptoethanol	0.78 ml
MilliQ H2O	9.22 ml

Heat Stable Recombinant Human FGF2 (25 µg/ml)

A	B
Heat Stable Recombinant Human FGF2	500 µg
0.1% BSA	20 ml

Final volume: 20 ml

hPSCs Medium + Rock inhibitor

A	B
hPSCs medium	500 ml
Y-27632 (1,000X)	500 µl

Final volume: 500 ml

Y-27632 (1,000X)

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A	B
Y-27632	5 mg
DMSO	1.56 ml

4 Change medium at day 3, 6, 8, 10, and then daily until colonies grow to medium size.

5 Perform Alkaline phosphatase (AP) staining as described in “Pluripotency markers staining” dx.doi.org/10.17504/protocols.io.b4yyqxxw

6 Image the entire stained well with ChemiDoc (Bio-Rad). Use a white cardboard as background.

7 Count AP positive colonies, N.

8 Calculate single cell survival rate by: N x dilution factor/500,000.
E.g. For the wells with seeding density 1/100, the dilution factor is 100.

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