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Single cell survival assay

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We use this protocol and it's working

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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.

Materials

	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**

	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)

	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.