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Cortical spheroid differentiation

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We use this protocol and it's working

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Abstract

This protocol describes the procedure of differentiating hPSCs into early cortical spheroids following an adaptation of a published protocol (Yoon et al 2019, Paşca et al. 2015).

Protocol overview

A. Media Formulations

B. Growth and Maintenance of Human Cortical Spheroids

Initial notes

A list of reagents and relevant vendor information can be found in the table listed under the materials tab.

Attachments



Cortical Spheroid Di...

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Materials

Reagent Table:

	Item	Vendor	Catalog Number
	DMEM-F12	Thermo Fisher	11320082
	DMEM With L-Glutamine and 4.5g/L Glucose; Without Sodium Pyruvate	Thermo Fisher	MT10017CV
	Knockout Serum Replacement	Gibco Life Technologies	10828028
	Newborn Calf Serum, USA origin, Heat Inactivated, sterile-filtered, suitable for cell culture	Sigma-Aldrich	N4762-500ML
	Penicillin-Streptomycin (10,000 U/mL)	Gibco Life Technologies	15140163
	MEM-NEAA (100x)	Gibco Life Technologies	11140050
	Gibco [®] GlutaMAX [®] Supplement	Gibco Life Technologies	35050061
	HEPEs buffer	Sigma-Aldrich	H0887-100ML
	Neurobasal [®] Medium	Gibco Life Technologies	21103049
	B-27 [®] Supplement (50X), minus vitamin A	Gibco Life Technologies	12587010
	Dorsomorphin, ≥98% (HPLC)	Sigma-Aldrich	P5499-5MG
	SB431542	Selleck Chemicals	S1067
	Y-27632 – ROCK Inhibitor	Chemdea	CD0141
	Recombinant Human BDNF Protein, CF	R&D Systems	11166-BD-050
	NT-3	Sigma	SRP3128
	Heat Stable Recombinant Human bFGF	Thermo Fisher Scientific	PHG0367
	Recombinant Human EGF Protein, CF	R&D Systems	236-EG-01M
	Dimethyl sulfoxide (DMSO)	Thermo Fisher Scientific	BP231-100
	Accutase	Thermo Fisher Scientific	SCR005
	DPBS w/o calcium and magnesium	Corning	MT21031CV
	Countess [™] Cell Counting Chamber Slides	Thermo Fischer Scientific	C10228
	AggreWell [®] 800 (6-well plate)	StemCell Technologies	34821
	Costar [®] 6-well Clear Flat Bottom Ultra-Low Attachment 6-well plate	Corning	3471



Media Formulations

1 hESC KSR Media (500 ml)

- 400 ml DMEM/F12
- 100 ml KSR
- 5 ml Pen-strep
- 5 ml NEAA
- 5 ml Glutamax
- 5 mL HEPES buffer

hESC Wash Media (500 ml)

- 500 ml DMEM
- 25mL ml Calf Serum
- 5 ml Pen-strep

hCS media (500 ml)

- 500 ml Neurobasal media
- 10 ml B27 (minus vitamin A)
- 5 ml pen-strep
- 5 ml Glutamax
- 5 mL HEPES buffer

Neural precursor expansion media

- 50 ml hCS media
- 40 μ l 25 μ g/ml FGF (final concentration, 20 ng/ml)
- 25 μ l 40 μ g/ml EGF (final concentration, 20 ng/ml)

Neural induction media

- 50 ml hESC KSR Media
- 50 μ l 10 mM SB431542 (final concentration, 10 μ M)
- 25 μ l 10 mM dorsomorphin (final concentration, 5 μ M)

Cortical differentiation media

- 50 ml hCS media
- 50 μ l 20 μ g/ml BDNF (final concentration, 20 ng/ml)
- 50 μ l 20 μ g/ml NT-3 (final concentration, 20 ng/ml)

Resuspend Dorsomorphin in DMSO at a concentration of 10 mM (1:2000)

Resuspend SB431542 in DMSO at a concentration of 10 mM (1:1000)

Resuspend Rock Inhibitor in DMSO at a concentration of 10 mM (1:1000)

Resuspend BDNF in water at a concentration of 20 μ g/ml (1:1000)



Resuspend NT3 in water a concentration of 20 µg/ml (1:1000)

Resuspend FGF at a concentration of 25 µg/ml (1:1250)

Resuspend EGF at a concentration of 40 µg/ml (1:2000)

Growth and Maintenance of Human Cortical Spheroids

35m

- 2 Grow stem cells feeder-free on 6-well plates as described in ([dx.doi.org/10.17504/protocols.io.b4mcqu2w](https://doi.org/10.17504/protocols.io.b4mcqu2w)).
- 3 On day -1 of differentiation remove hESC Media and feed with hESC KSR Media + 10uM Rock Inhibitor (1:1000).
- 4 Pre-coat an aggrewell plate according to manufacturer instructions.
- 5 On day 0 of differentiation, aspirate media and add  1 mL of PBS- onto each well.
- 6 Immediately Remove PBS- and add  1 mL of Accutase (1:3 Diluted with PBS-) onto each well of the 6 well plate.
- 7 Return plate to incubator for ~  00:30:00 or until cells are dissociated into single cell suspension. 30m
- 8 Remove plate from incubator. Pool the cells into a 15mL conical tube per plate (6mL total volume).
- 9 Using  6 mL hESC Wash media, rinse the wells and dilute the cell-Accutase suspension.
- 10 Spin down cells for  00:05:00 at  200 x g to  300 x g . 5m
- 11 Remove the supernatant from the falcon tube using a sterile glass pipette. Be careful not to aspirate any of the cells.
- 12 Resuspend the cells in  5 mL of hESC KSR Media + 10uM Rock Inhibitor (1:1000).



- 13 Using a 5mL serological pipette, filter cells through a 40 µm cell strainer into a new 50 ml conical tube.
- 14 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.
- 15 Count cells with Countess automated cell counter or hemocytometer, averaging the counts from the two sets.
- 16 Resuspend the cells to a concentration of 18 million cells per 5 mL of media (3.6 million cells per mL) in hESC KSR Media + 10µM Rock Inhibitor (1:1000).
- 16.1 If needed, combine tubes from multiple plates and re-concentrate by spinning down at  200 x g to  300 x g and resuspending in an appropriate volume of hESC Media + Rock Inhibitor 1:1000.
- 17 Transfer  5 mL of suspension to one well of a pre-coated 6-well Aggrewell 800 plate. Return plate to the incubator.
- 18 The next day (Day 1), prepare neural induction media.
- 19 Remove the aggrewell plate from the incubator and use a serological pipette to transfer the newly formed Embryoid Bodies (EBs) to a 15 ml conical tube.
- 20 Allow EBs to settle (1-2 mins, but check before aspirating to ensure EB retention) and aspirate off media, taking special care to not suck up the EBs.
- 21 Resuspend in 5 ml of neural induction media and deposit into one well of a 6-well ultra-low attachment plate.
- 22 Repeat steps 17-20 to change media each day until Day 6.
- 23 On Day 6, remove cell suspension from the plate and deposit into a 15 ml falcon tube
- 24 Allow spheroids to settle to the bottom of the tube (~1-2 mins).



- 25 While settling, make neural precursor expansion media.
- 26 Remove the supernatant from the settled spheroid suspension.
- 27 Resuspend spheroids in neural precursor expansion media.
- 28 Return to the Ultra-Low Attachment plate and return the plate to the incubator.
- 29 Repeat steps 22-27 to feed cells every day until Day 16 and then every other day until Day 25
- 30 On day 25, prepare Cortical differentiation media.
- 31 Remove cell suspension from the plate and deposit into a 15 ml falcon tube.
- 32 Allow spheroids to settle to the bottom of the tube (~1-2 mins).
- 33 Remove the supernatant from the settled cell suspension.
- 34 Resuspend cells in Cortical differentiation media.
- 35 Return to the Ultra-Low Attachment plate and return the plate to the incubator.
- 36 Repeat steps 30-34 every four days (d29, 33, 37, 41) day until Day 43
- 37 On day 43, change the media as above, but replace with only hCS media with no additives.



- 38 Change media by sedimenting the spheroids, removing the supernatant, resuspending in hCS Media, and returning to the Ultra-Low Attachment plate every four days until the termination of the experiment.