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Survival factors for hPSC growth

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Tamer Onder¹, Valeria Fernandez Vallone², Lyn Healy³, Nathalie Lefort⁴, Katarzyna Ludwik², Lisa Pavinato^{5,6}, Fatma Visal OKUR⁷, Harald Stachelscheid²

¹Koc University;

²Core Unit pluripotent Stem Cells and Organoids - Berlin Institute of Health @ Charite, Berlin, Germany;

³The Francis Crick Institute;

⁴Université de Paris, Imagine Institute, iPSC Core Facility, INSERM UMR U1163, F-75015 Paris, France.;

⁵Institute of Oncology Research (IOR), Bellinzona Institutes of Science (BIOS+), Bellinzona, Switzerland;

⁶Faculty of Biomedical Sciences, Università della Svizzera Italiana, Lugano, Switzerland.;

⁷Hacettepe University, Center for Stem Cell Research and Development (PEDISTEM) and Hacettepe University Faculty of Medicine, Department of Pediatrics

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Harald Stachelscheid

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We use this protocol and it's working

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Abstract

This protocol describes the preparation and supplementation of hPSC culture media with survival factors.



Materials

LABORATORY EQUIPMENT AND CONSUMABLES

Use sterile material

- 1/5/10 mL pipettes
- 15/50 mL conical tubes
- 10/200/1000µL tips and micropipettes
- 0.5 or 1.5 mL tubes
- Centrifuge
- Vortex
- Class II Biosafety Cabinet

MEDIA AND REAGENTS

⊗ Y-27632 Selleckchem Catalog #S1049

⊗ Y-27632 dihydrochloride MedChemExpress Catalog #HY-10583

⊗ Y-27632 (Dihydrochloride) 5 × 10 mg STEMCELL Technologies Inc. Catalog #72307

⊗ • Chroman I MedChemExpress Catalog #HY-15392

⊗ Emricasan (IDN-6556) Selleckchem Catalog #S7775

⊗ ISRIB (trans-isomer) MedChemExpress Catalog #HY-12495

⊗ Trans-ISRIB Tocris Catalog #5284

⊗ Polyamine Supplement (1000×) Merck MilliporeSigma (Sigma-Aldrich) Catalog #P8483

⊗ Dimethyl sulfoxide (DMSO) Merck MilliporeSigma (Sigma-Aldrich) Catalog #D2650

⊗ CloneR2 STEMCELL Technologies Inc. Catalog ## 100-0691

hPSC culture conditions and survival factors choice depend on hPSC line and individual lab practices.

For options refer to protocols:

Maintenance of hPSC

Coating of tissue Culture Vessels for hPSC

- 1 Select survival factor according to the desired culture condition or lab-workflow from the dropdown below.

Note

Presence of survival factors in the culture medium can have strong effects on hPSC morphology. Refer to **Reference pictures of hPSC cultured in defined conditions** for examples of typical morphologies.

STEP CASE

Y-27632 ROCK inhibitor 7 steps

Stock solution preparation and aliquoting

40m

- 2 Spin down the vial containing ROCK inhibitor Y-27632 (ROCKi) powder prior to opening.

2m

Stock solution preparation and aliquoting

40m

- 3 Reconstitute in required volume of sterile water in a 15 mL Falcon tube. Refer to **Table 1** for quantity and corresponding volume.

5m

	A	B	C
	Amount of ROCKi	Volume of water	Final concentration
	1mg	625ul	5 mM (500x)
	5mg	3.125ml	5 mM (500x)
	10mg	6.25ml	5 mM (500x)
	50mg	31.25ml	5 mM (500x)

Table 1. Guidance to volumes needed to dissolve different amounts of ROCKi.

**Note**

Alternatively, use DMSO as solvent.

ROCKi solubility in both water and DMSO is maximum [M] 100 millimolar (mM)

- 4 Prepare  200 μL aliquots in 0.5 mL eppendorf tubes.

15m

Note

If ROCKi is provided as ready-to-use solution e.g. [M] 10 millimolar (mM) , prepare

 50 μL - 100 μL aliquots and store as described below.

- 5 Store aliquots at  $-20\text{ }^{\circ}\text{C}$ or at  $-80\text{ }^{\circ}\text{C}$ for long-term storage (up to two years).

2m

Preparation of culture medium supplemented with ROCKi

5m

- 6 Thaw required number of ROCKi aliquots according to culture media volume needed.

2m

Note

After thawing, ROCKi aliquots can be stored at  $4\text{ }^{\circ}\text{C}$ for up to two weeks.

Do not repeatedly freeze-thaw this reagent.

- 7 Supplement culture media to the final concentration of [M] 10 micromolar (μM) ROCKi (**Table 2**).

3m

	A	B	C
	Volume Medium [mL]	Volume ROCKi 5mM [μL]	Volume ROCKi 10mM [μL]
	5	10	5



	A	B	C
	12	24	12
	50	100	50
	500	1000	500

Table 2. ROCKi volume per culture media volume supplemented accorsing to ROCKi stock concentration.

8 Always supplement fresh the culture media with ROCKi prior to use.

Proceed with cell culture, refer to protocols:

[Maintenance of hPSC](#)

[Single cell passaging of hPSC](#)

[Reference pictures of hPSC cultured in defined conditions](#)

Protocol references

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