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Coating of tissue Culture Vessels for hPSC

In 1 collection

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We use this protocol and it's working

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Disclaimer

The reported protocols are based on the authors experience, and may partially differ from the original protocols provided by the companies.

Abstract

This protocol describes procedures to coat culture vessels for the maintenance of hPSC using different extracellular matrices.

Materials

LABORATORY EQUIPMENT AND CONSUMABLES

Use sterile material

- 1/5/10 mL pipettes
- 1.5/15/50 mL conical tubes
- 10/200/1000µL tips and pipettes (optional)
- Cell culture treated plastic vessels of choice e.g. 24, 12 or 6-well plates, T25, T75 flasks, 10cm dishes
- Aspirator pump with disposable pipette
- Class II Biosafety Cabinet
- Wet ice

MEDIA AND REAGENTS

Vitronectin options tested by authors

- Vitronectin (VTN-N) Recombinant Human Protein, Truncated **Thermo Fisher Catalog #**A14700 (500 µg/ml)
- Vitronectin XF™ **STEMCELL Technologies Inc. Catalog #**#07180 (250 µg/ml)
- Recombinant Human Vitronectin Protein, CF **R&D Systems Catalog #**2308-VN

Geltrex options tested by authors

- Geltrex LDEV Free hESC Quality 5 ml **Thermo Fisher Scientific Catalog #**A1413302
- Geltrex™ hESC-Qualified, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix **Thermo Fisher Catalog #**A1569601

Matrigel options tested by authors

- Growth Factor Reduced (GFR) Matrigel® phenol red-free **Corning Catalog #**356231
- Matrigel hESC-Qualified Matrix, LDEV-free **Corning Catalog #**354277

Laminin options tested by authors

- BIOLAMININ 521 LN (LN521) **Biolamina Catalog #**LN521
- iMatrix-511 Recombinant Laminin E8 Fragments (Laminin matrix) **amsbio Catalog #**AMS.892 011

Diluent

- DPBS (no Ca, no Mg) **Thermofisher Catalog #**14190144 or other vendors
- Gibco™ DPBS (10X), calcium, magnesium **Fisher Scientific Catalog #**14080055
- DMEM/F12 **Thermo Fisher Scientific Catalog #**11320033 or
  Advanced DMEM/F-12 **Thermo Fisher Catalog #**12634028
- CellAdhere™ Dilution Buffer **STEMCELL Technologies Inc. Catalog #**07183 can be used instead of DPBS in combination with Vitronectin XF™ **STEMCELL Technologies Inc. Catalog #**#07180 (see "Guidelines and Warnings").

	A	B	C	D	E
	Product	**Company**	**Catalogue number**	**Stock concentration**	**Storage upon receipt**
	Vitronectin (VTN-N) Recombinant Human Protein, Truncated	ThermoFisher	A14700	500 µg/mL	-80°C
	Vitronectin XF	StemCell Technologies	07180	250 µg/ml	-20°C to -80°C
	Recombinant Human Vitronectin Protein, CF	R&D Systems	2308-VN	50 µg	-20°C to -80°C
	Geltrex LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix	ThermoFisher	A1413302	12 to 18 mg/ml	-20°C to -80°C
	Geltrex hESC-Qualified, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix	ThermoFisher	A1569601	0.12 to 0.18 mg/mL	2°C to 8°C protected from light
	Corning Matrigel Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-free, LDEV-free	Corning	356231	batch dependent	-20°C to -80°C
	Corning Matrigel hESC-Qualified Matrix, LDEV-free	Corning	354277	batch dependent	-20°C to -80°C
	Laminin 521	Biolamina	LN521-05	100 µg/ml	-80°C
	iMatrix-511	amsbio	AMS.892011	500 µg/ml	2°C to 15°C

Table 4. List of tested coatings, their initial concentration and the temperature of storage upon reception.

Safety warnings

Vitronectin coating

The use of Non-tissue culture-treated cultureware **STEMCELL Technologies Inc.** and CellAdhere™ Dilution Buffer 100 mL **STEMCELL Technologies Inc. Catalog #**7183 is recommended with Vitronectin XF™ **STEMCELL Technologies Inc. Catalog #**#07180 from the vendor. However, we tested the use of normal tissue culture-treated cultureware and DPBS no calcium, no magnesium **Invitrogen - Thermo Fisher Catalog #**14190136 with Vitronectin XF™ **STEMCELL Technologies Inc. Catalog #**#07180 , and no major differences were noted. In the protocol, we refer to this last combination.

1 Select extracellular matrix (ECM) according to desired culture conditions. See protocol **Maintenance of hPSC** for an overview on medium/matrix combinations that have been validated by CorEuStem members. 5m

Refer to **Table 1** for final volumes needed according to vessel format.

	A	B	C
	Culture vessel	**Approximative growth area (cm2)**	**Volume of diluted matrix per vessel/well (ml)**
	96 well	0.32	0.05-0.1
	48 well	1.1	0.1-0.2
	24 well	1.9	0.2-0.5
	12 well	3.5	0.4-1.0
	6 well	9.6	1.0-2.0
	100 mm dish	56.7	5.0-8.0
	T25 flask	25	2.5
	T75 flask	75	7.5
	T175 flask	175	10-18

Table 1. Suggested volumes of diluted matrices for coatings.

STEP CASE

Vitronectin 7 steps

Different versions of Vitronectin are reported in "Materials" section.

Aliquoting and storage of Vitronectin 15m

2 Upon receipt, aliquot Vitronectin and store vials according to manufacturer instructions (**Table 2**). 15m

	A	B	C	D
		A14700 ThermoFisher	**07180 StemCell Technologies**	**2308-VN R&D Systems**

	A	B	C	D
	Thawing of stock	RT	RT or ON 4°C	Powder
	Reconstitution	Ready-to-use 500µg/mL	Ready-to-use 250µg/mL	50 µg in 200 µl sterile DPBS to obtain 250µg/mL
	Aliquot size*	60µl/vial	120µl/vial	120µl/vial
	Storage of aliquots	-80°C	-20°C/-80°C	-80°C

Table 2. Instructions describing reconstitution and aliquoting of vitronectin variants from different manufacturers
**Prepare aliquots according to your needs: the format we indicated for the aliquoting is enough to coat a full 6-well plate when diluted.*
RT = room temperature; ON = over night

Do not store aliquots in frost-free freezer. Do not repeatedly freeze and thaw.

Coating

1h 31m

3 Thaw a vial of Vitronectin at Room temperature for 00:02:00 . 2m

4 In a 15-ml tube, dilute the Vitronectin in 6 mL DPBS, gently pipetting up and down to properly resuspend it. 2m

Note

The indicated dilutions are for a final concentration of [M] 5 µg/mL . Vitronectin coating has been tested and validated up to a final concentration of [M] 3.33 µg/mL for hPSC maintenance. **Optimal concentration depends on specific hPSC lines, the application or research objectives.**

5 Dispense diluted Vitronectin on the preferred dish, being careful that the surface is completely covered. If necessary, rock or tap the plate to facilitate this process. Refer to **Table 1** for suggested volumes according to vessel format. 2m

6 Incubate the coated tissue-culture vessels for 00:30:00 to 01:00:00 at Room temperature . 1h

7 After incubation, aspirate the Vitronectin solution right before use, by tilting the plate and ensuring not to scrape the bottom of the well. Directly proceed with the plating of cells. Refer to **Maintenance of hPSC**, and/or to **Single cell passaging of hPSC**, **Non-enzymatic passaging of hPSC**. Do not allow the coated surface to dry. 5m

Note

Vitronectin-coated plates cannot be re-used: always seed cells onto fresh Vitronectin-coated plates.

8 If not used immediately, coated plates can be stored at 2 °C to 8 °C up to seven days. 20m

Note

In our experience, the coating can be stored up to 30 days; however this is not suggested by the manufacturers.

For stored plates, prior to plating the cells, allow plates to equilibrate at Room temperature for about 00:20:00 and go to step #9

Note

Do not allow the coated surface to dry out. To prevent excessive evaporation of the coating, tightly seal the plates with Parafilm. Additionally, some DPBS may be added after the incubation time is finished.

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