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Human Truncated Vitronectin / VTN-N Protein (Cat. No. VIN-H5117) hPSC Coating Culture Protocol

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1. Experimental instruments and materials

1.1 Instruments: Biosafety cabinet, cell incubator, low temperature horizontal centrifuge, inverted microscope, EnSight (PerkinElmer, HH3400), Microscopic, Flow cytometer.

1.2 Materials: Sterile pipette tips, Sterile EP tube and other consumables.

1.3 Reagents:

Name	Vendor	Cat. No.
mTeSR™-Plus	STEM CELL	100-0276
Y27632	MCE	HY-10071
Gentle Cell Dissociation Reagent	STEM CELL	100-0485
PBS	HyClone	SH30256.01
Cell Counting-Lite 2.0 Luminescent Cell Viability Assay	Vazyme	DD1101-02
24 well plate	Nest	702001
OCT4	CST	2750S
NANOG	CST	4903S
SOX2	Santa Cruz	sc-365823
PE-OCT4	Biolegend	653703
PE-SOX2	Biolegend	656103
SSEA4	CST	4755T

1. Experimental instruments and materials

1.4 Activity Assay :

Cell Viability Assay: CellCounting-Lite 2.0 Luminescent Cell Viability Assay

FACS Assay **:** OCT4, SOX2, SSEA4

IF Assay **:** OCT4, NANOG, SOX2

1.5 Test samples & concentration:

vitronectin： 0.5μg/cm^2

1.6 Groups

ACRO: Human Truncated Vitronectin / VTN-N Protein (Cat. No. VIN-H5117)

2. Vitronectin plate coating

2.1. Use sterile PBS to dissolve vitronectin and configure into a 100 μg/ml solution. The final working concentration of **0.5-1 μg/cm²** is recommended. When culture hPSCs for the first time, a higher working concentration is recommended to adapt the new matrix. We recommend using an initial coating concentration of 0.5 μg/cm² on the culture surface. The detailed guidance for calculating the adding volumes of vitronectin is referred to the Table.

Culture vessel	Surface area	Coating concentration	Volume (vitronectin-100μg/ml)	mTeSR™-Plus
6-well	10 cm²	0.5 μg/cm²	50 μL/well	2 mL
12-well	4 cm²	0.5 μg/cm²	25 μL/well	1 mL
24-well	2 cm²	0.5 μg/cm²	10 μL/well	0.5 mL
48-well	1 cm²	0.5 μg/cm²	5 μL/well	0.25 mL

For example, for one well of a 12-well plate, add 20μL of vitronectin (2μg) in 480μL PBS. Then, add 0.5 mL of diluted vitronectin solution to the well.

2.2. Add indicated volumes of the vitronectin-PBS mixture into the each well and gently shake to ensure that matrix is spread across the well.

2.3. Incubate at room temperature for at least 1 hour before use. Do not allow the culture vessel to dry.

2.4. Aspirate the vitronectin solution and discard when cells are ready to be plated.

3. Cell Culture - hPSC

3.1. Pre-warm the required volume of cell culture medium containing 10 μM Y-27632 (final concentration) to 37° C.

3.2. Remove the spent culture medium and wash the adherent cells gently with 0.5 ml of PBS in each well (12 well).

3.3. Add 0.5ml Gentle Cell Dissociation Reagent to each well. Stand at room temperature for 6-8 minutes or keep under microscopic observation until cells are not bound to the plate.

3.4. Add equal volume of mTeSR™-Plus, gently mix the cells and transfer it into a centrifuge tube at room temperature at 300g for 5 minutes.

3.5. Adjust the concentration of the cell suspension with the pre-warmed medium containing 10 μM Y-27632. The recommended cell density is 8×10^4 cells/well in a 12-well plate, and ensure passage in 4-5 days.

3.6. When cells are cultured to passage 5, cells are collected for detection of stemness genes.

Culture vessel	6 well	12 well	24 well	96 well
Cell numbers	$2.5\sim3.5\times10^5$	$6\sim8\times10^4$	$3\sim4\times10^4$	$0.5\sim1\times10^4$

4. Cell Counting-Lite 2.0 Luminescent Cell Viability Assay

4.1. Pre-warm the 1X Cell Counting-Lite 2.0 Luminescent to room temperature.

4.2. Add 250µl 1X Cell Counting-Lite 2.0 Luminescent to 24-well cell culture plate and incubate at room temperature for 15 min.

4.3. Transfer 250µl supernatant to a black dark plate and test on the EnSight (PerkinElmer, HH3400).

5. FACS

5.1. Collected cells and add 4% paraformaldehyde to fix for 15 min at room temperature.

5.2. Add 500µl PBS to wash and incubate at room temperature for 15 min with 350µl 0.5% Triton X-100.

5.3. Wash with 500µl PBS after centrifugation and incubate at 4° C with 2% BSA containing antibodies (PE-OCT4, PE-SOX2, SSEA4) for 1h in the dark.

5.4. Transfer the cells to a 96-well V-plate with 100µl PBS ready for flow cytometry.

6. IF

6.1. Discard the supernatant and add 350µl of 4% paraformaldehyde to fix for 15 min at room temperature (for 24-well plate).

6.2. Wash with 500µl PBS and incubate at room temperature with 350µl 0.5% Triton X-100 for 15 min.

6.3. Wash with 500µl PBS and incubate at room temperature with 2% BSA for 1h.

6.4. Wash with 500µl PBS and incubate overnight at 4℃ with 2% BSA coating primary antibody (OCT4, NANOG, SOX2).

6.5. Wash 3 times with 500µl PBS and incubate at room temperature with 2% BSA coating second antibody.

6.6. Wash 3 times with 500µl PBS and incubate at room temperature with PBS coating DAPI for 10min.

6.7. Wash 3 times with 500µl PBS and add 100µl PBS ready for Microscopic observation.

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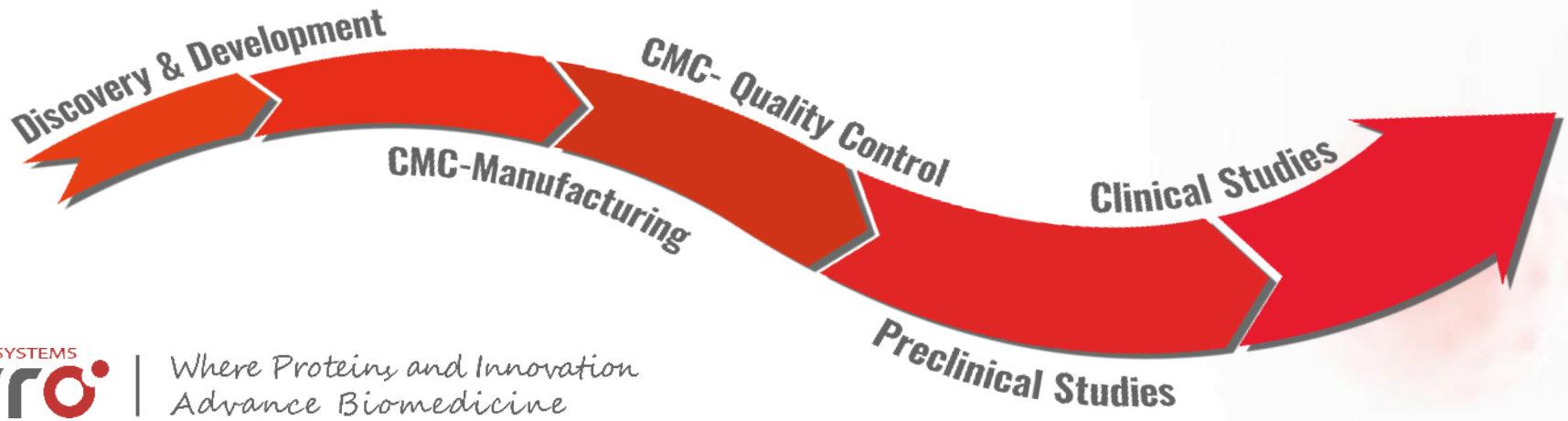

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