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Recombinant Fibronectin Fragment, Premium Grade (Cat. No. FIN-H5116) Stem Cell Culture Protocol

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1. Experimental instruments and materials

1.1 Instruments: Biosafety cabinet, cell incubator, low temperature horizontal centrifuge, inverted microscope.

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

1.3 Reagents:

Name	Vendor	Cat. No.
StemPro™ MSC SFM	Thermo Fisher SCIENTIFIC	A1033201
GlutaMAX™	Thermo Fisher SCIENTIFIC	35050061
TrypLE™ Select	Thermo Fisher SCIENTIFIC	12563029
PBS	hyClone	SH30256.01
CellCounting-Lite 2.0 Luminescent Cell V	Vazyme	DD1101-02

1.4 Human fibronectin activity assay: CTG, FACS

1.5 MSC: MSCs are derived from cord blood tissue, and cells were cultured to passage 3 before the experiment.

1.6 Working concentration:

Recombinant Fibronectin fragment, premium grade (Cat. No. FIN-H5116) : 0.5μg/cm2

2. Experimental contents and methods

2.1. Use sterile PBS to dissolve fibronectin and configure into a 100 μg/ml. The final working concentration of **0.5-1 μg/cm²** is recommended.

The detailed guidance for calculating the adding volumes of fibronectin is referred to the Table.

In this experiment, the concentration of fibronectin was **0.5 μg/cm²** for 24-well plate.

2.2. Add 400μl fibronectin-PBS mixture into the each well and gently shake to ensure that matrix is spread across the 24-well.

2.3. Transfer the plate into a 37° C incubator for incubation overnight. The minimal amount of time for use is 1 hours.

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

Culture vessel	Surface area	Coating concentration	Volume（Fibronectin）	Volume（PBS）	Total volume	StemPro™ MSC SFM
6-well	10 cm^2	0.5 μg/cm^2	50 μL/well	1950 μL/well	2 mL/well	2 mL
12-well	4 cm^2	0.5 μg/cm^2	20 μL/well	780 μL/well	0.8 mL/well	1 mL
24-well	2 cm^2	0.5 μg/cm^2	10 μL/well	390 μL/well	0.4 mL/well	0.5 mL
T25	25 cm^2	0.5 μg/cm^2	125 μL/well	4.875 mL/well	5 mL/well	5 mL
T75	75 cm^2	0.5 μg/cm^2	375 μL/well	14.625 mL/well	15 mL/well	15 mL

3. Cell Culture - MSC

3.1. Discard the culture supernatant and rinse with 1mL PBS (6-well).

3.2. Discard the PBS and add 1mL TrypLE™ Select. Stand at room temperature for 30-60 seconds or keep under microscopic observation until cells are not bound to the plate.

3.3. Add equal volume of StemPro™ MSC SFM, gently mix the cells and transfer it into a centrifuge tube at 300g for 5 minutes.

3.4. Discard the supernatant and resuspend cells with StemPro™ MSC SFM.

3.5. Transfer $2*10^4$ cells with adequate volume of fibronectin to 24-well plate. Shake the plate gently to evenly distribute cells.

The detailed guidance for calculating the adding volumes of fibronectin is referred to the Table.

3.6. Medium was changed every 3 days, and tested samples at 4 days for CTG of passage 1 and passage 3. And collected samples for flow cytometry after 4 days of passage 3.

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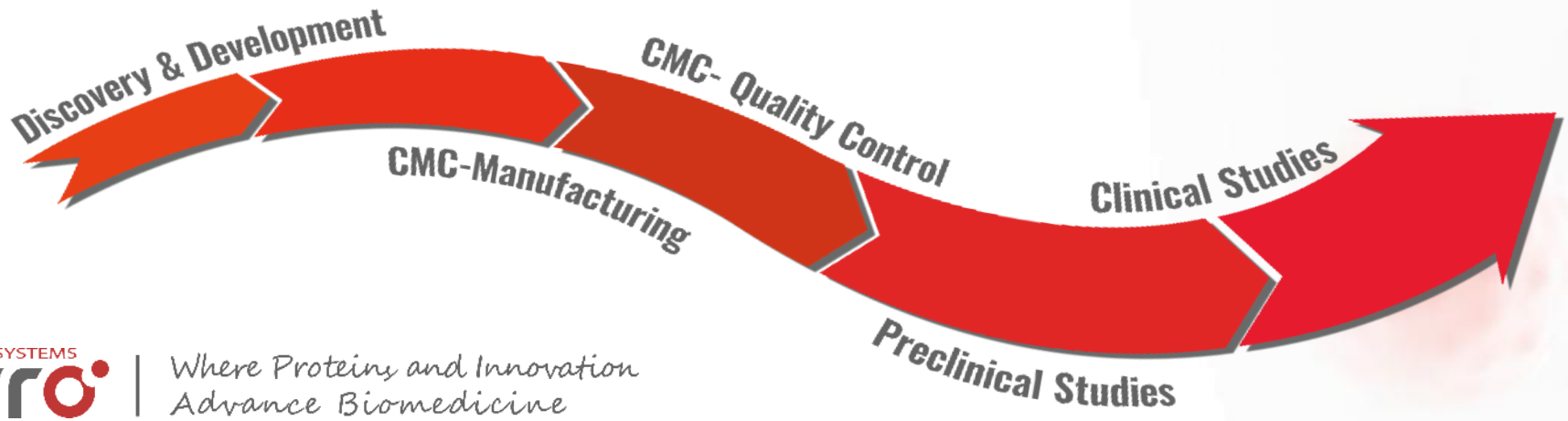

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