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STC-1 Passaging and PD Associated Microbe Stimulation Protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

STC-1 Passaging and PD Associated Microbe Stimulation Protocol

Materials

Complete Medium
DMEM, high glucose, no glutamine
4mM L-glutamine
1mM sodium pyruvate
5% FBS

Other Reagents
0.05% Trypsin-EDTA
1X D-PBS without calcium and magnesium
75cm2 Flask
12 well plates

Troubleshooting

Passaging & Plating Protocol

1 Cells are passaged when they reach approximately 70% confluence

Warm trypsin, 1XPBS, and complete medium in water bath for 15 min.

2 Aspirate off culture medium

3 Rinse cells with 10mL D-PBS

4 Add 3mL Trypsin to flask. Place flask back into 37°C/5% CO2 incubator for 7 minutes.

5 Observe cells under microscope to ensure they have detached from the bottom of the flask.

a. These cells are particularly sticky, so giving them a nice tap may be necessary to detach them.

6 Add 9mL of complete medium to flask and transfer cell suspension to a 15mL tube.

7 Spin down at 130g for 5 min

8 Aspirate off supernatant and resuspend cell pellet in 1mL culture medium.

9 Count cells

10 Prepare cell suspension at a concentration of 3×105 cells/mL

11 Add 1mL cell suspension per well in 12 well plate(s)

12 For PD associated microbe stimulation, allow cells to rest for approximately 24 hours,

13 Treat STC-1s with 10 colony forming units (CFU) of PD associated microbe per STC-1 cell

14 Incubate for desired time and then collect conditioned media and/or cells for downstream analyses

LPS Pre-Treatment

15 Following plating, allow cells to rest for 24 hours

16 Treat cells with 10ng/mL LPS for 24 hours

17 24 hr after LPS treatment, stimulate cells with 10 CFU of PD associated microbes per STC-1 cell

18 Incubate for desired time and then collect conditioned media and/or cells for downstream analyses