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# STC-1 Passaging and PD Associated Microbe Stimulation Protocol

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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

# OPEN ACCESS



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

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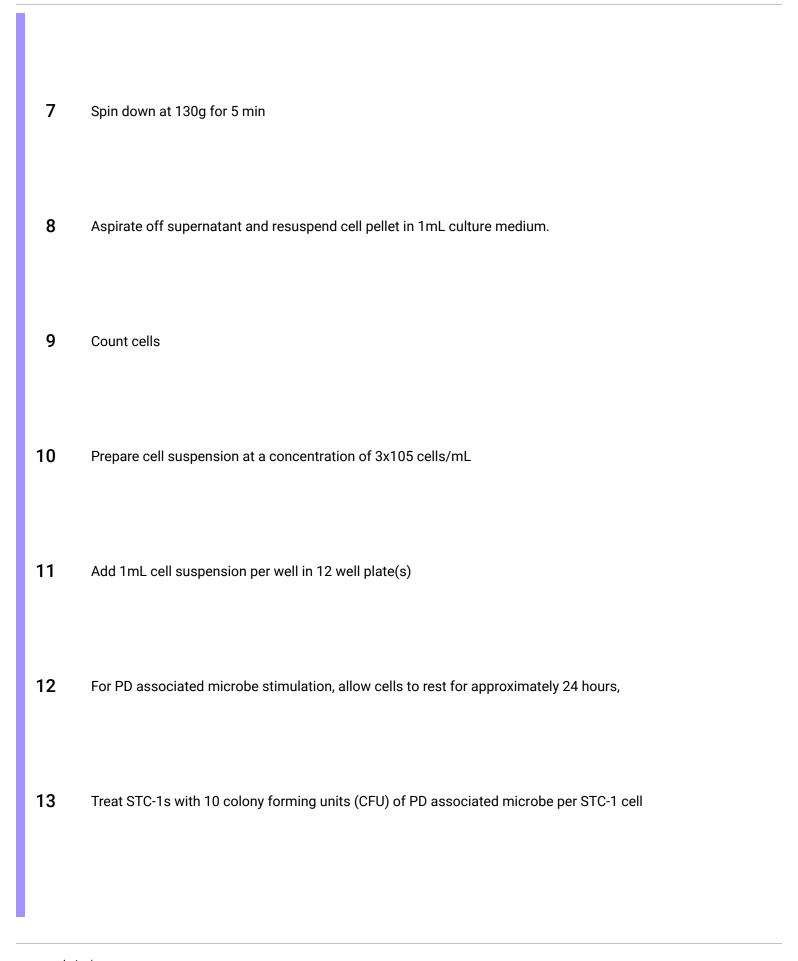
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1 Cells are passaged when they reach approximately 70% confluence

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.



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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
- 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