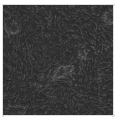

Mammalian Cell Culture: Subculturing

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481b Laboratory

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ABSTRACT

This protocol details how to subculture nearly confluent mammalian cells grown in a T-25 flask.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS TEXT

- Gloves
- 0.05% or 0.25% warmed Trypsin-EDTA
- Warmed cell culture Media (e.g. DMEM:F12, EMEM)
- DPBS
- 15 mL centrifuge tube
- Serological pipette and tips
- 1000 µL pipette and tips

SAFETY WARNINGS

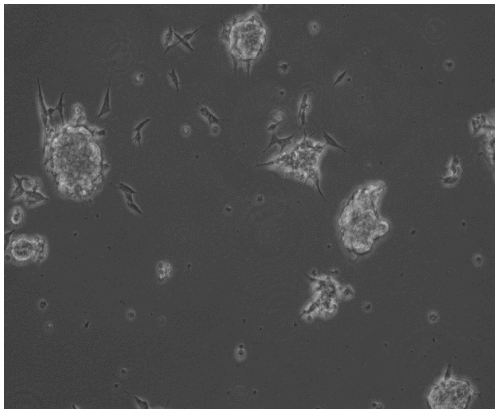
Lab coat and gloves must be worn at all times.

Assess Cell Confluency

1 Under light microscope, look at the cells and assess level of confluency. This is how you will determine the need to subculture.

NOTE

Confluency can be estimated by evaluating the percentage of surface covered by cells.



Low confluency SH-SY5Y

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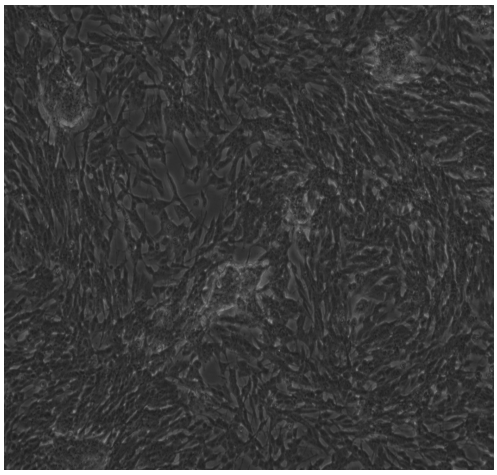

High confluency SH-SY5Y

Wash Cells

2 Using serological pipette, add 1 ml DPBS to T-25 flask.

3 Using serological pipette, remove DPBS and dispose into wase beaker.

4 Repeat the above 2 steps, so that you will wash the cells twice.

NOTE
Always use a fresh pipette tip when drawing liquid from a stock solution.

Trypsinize

5 Add 1 ml warmed trypsin-EDTA to T-25 flask.

6 Wait 00:05:00 for trypsin-EDTA to detach the cells.

NOTE
This time will vary in practice, and depends on cell type and trypsin concentration (i.e. 0.05% vs 0.25%). Some cell types may take up to 15 minutes. In those cases, assess detachment progress using a light microscope.

7 Add 1 ml cell culture media.

NOTE
Trypsin-EDTA is neutralized by adding a volume of cell culture media equal to that of trypsin-EDTA.

Spin Down

8 Using a serological pipette, transfer the cell suspension (cells, trypsin-EDTA, and cell culture media) into a 15 mL centrifuge tube.

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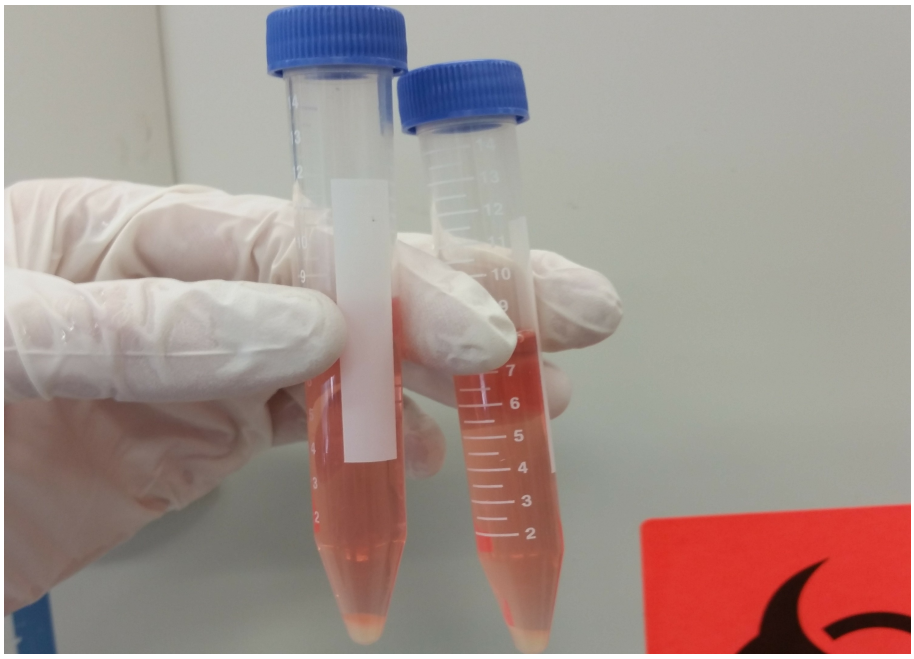
9 Add 3.5 ml fresh cell culture media to T-25 flask, this will preserve any remaining cells.

10 Centrifuge the cell suspension on 1.5 kRPM for 00:03:00 .

NOTE
This has been perfomed by T.A. ahead of time. Bring your cell suspension to trade for a spun down cell pellet.



Centrifuge tubes with large cell pellets at the bottom and 8 mL of supernatant.

Resuspend and Reseed

11 Remove supernatant, dispose into waste beaker.

NOTE
You can leave a small amount with the serological pipette, the rest will be taken off in the next step.

12 Using a 1000 µL pipette, carefully remove the remaining supernatant, being cautious not to disturb the cell pellet.

SAFETY INFORMATION
Always dispose of pipette tips in sharps container. Do not use the same tip twice.

13 Add 1000 µl cell culture media to the cell pellet, and allow to sit for 00:01:00 .

14 Gently pipette mix the cell pellet until the pellet is resuspended.

NOTE
Pipette mixing is done by slowly drawing in solution and pushing it out several times, all without removing the pipette tip from the solution. Ask T.A. for help on this if you need some pointers.

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15 Reseed your T-25 flask by transferring 500 µl cell suspension to the flask.

NOTE

In practice you would reseed the other 500 µl into another flask, or even split the cell suspension into 3 or 4 flasks. Alternatively, you make take the remaining cell suspension for an experiment.

Incubate

16 Incubate at 37 °C in CO_2 incubator.

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