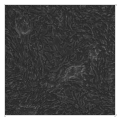

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Mammalian Cell Culture: Subculturing

Version 2

Forked from Mammalian Cell Culture: Subculturing

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ABSTRACT

This protocol details how to subculture/passage nearly confluent mammalian cells grown in a tissue culture flask.

GUIDELINES

- Gloves must be worn at all times.
- Perform all tasks within biosafety cabinet.
- Anything entering biosafety cabinet must be generously sprayed with 70% ethanol (even you).
- When finished, wipe biosafety cabinet with 70% ethanol, and UV for at least 15 minutes.

MATERIALS TEXT

- Cultured T-75 [or T-25] flask
- Gloves
- 0.05% or 0.25% Trypsin-EDTA
- Cell culture Media (e.g. DMEM:F12, EMEM)
- DPBS
- 15 mL centrifuge tube
- Serological pipet and tips
- 1000 µL pipette and tips
- Waste beaker

SAFETY WARNINGS

Gloves must be worn at all times. Perform all work within biosafety cabinet.

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.

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

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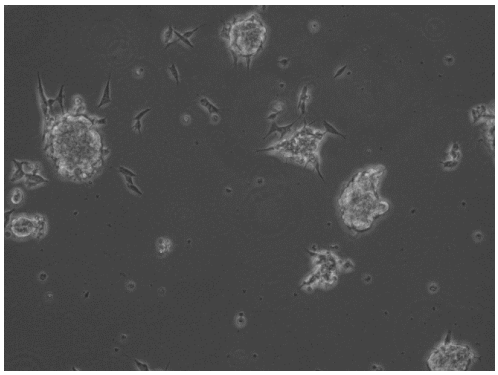
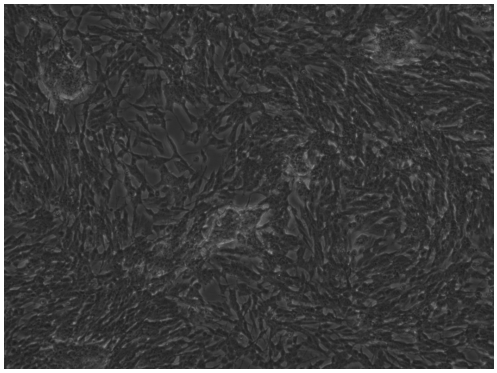


Low confluency SH-SY5Y



High confluency SH-SY5Y

Wash Cells

2 Remove media from flask.

3 Using serological pipette, add **4 ml** DPBS to flask. [**1 ml** for T-25]

4 Using serological pipette, remove DPBS and dispose into waste beaker.

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

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

Trypsinize

6 Add **4 ml** warmed trypsin-EDTA to T-25 flask. [**1 ml** for T-25]

7 Wait approximately **00:05:00** for trypsin-EDTA to detach the cells.

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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.

For cell types that take longer to detach, place flask in incubator to keep the temperature high enough for trypsin to remain active.

8 Add **4 ml** cell culture media. [**1 ml** for T-25]

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

Spin Down

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

10 Add **9.5 ml** fresh cell culture media to T-25 flask, this will preserve any remaining cells.

11 Centrifuge the cell suspension on **1500 rpm** for **00:03:00** .

Resuspend and Reseed

12 Remove supernatant, dispose into waste beaker.

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

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

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

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

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

Pipette mixing is done by slowly drawing in solution and pushing it out several times, all without removing the pipette tip

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from the solution. Ask T.A. for help on this if you need some pointers.

16 Seed 2 flasks each with **500 µl** cell suspension.

You may seed more than 2 flasks, just use smaller volumes in each.

17 Label flask with updated passage number along with the date.

Incubate

18 Incubate at **37 °C** in CO_2 incubator.

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