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Adherent mammalian Cell Culture maintenance

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We use this protocol and it's working

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Abstract

Subculturing for HEK293T, SH-SY5Y and U2OS cells.

Materials

DMEM base media (Fisher Scientific, Cat# 11-965-118)
10% heat inactivated fetal bovine serum (FBS, Fisher Scientific, Cat# 10438026)
1% penicillin/streptomycin (Gibco, Cat# 15140-122)
0.05% Trypsin-EDTA (Gibco, Cat# 25300-062)

Preparations

1 ▪ Culture Media:
Use appropriate culture media for your cells. For most mammalian cell lines (for example HEK-293T, SH-SY5Y, U2OS) DMEM base media supplemented (500 ml) with 10% FBS (50 ml, heat inactivated) and optionally 1% Antibiotics (f.ex. Penicillin/Streptomycin, 5 ml) work well. Add everything to the DMEM media and incubate the bottle in 37°C prior usage for approx. 15-30 min.

Note: The mixed media can be stored at 4°C for several weeks, up to a month.

▪ Trypsin-EDTA:
Most cells will detach with 0.05% Trypsin-EDTA, but for some cell lines 0.25% will work better. Place one aliquot in 37°C water bath for 15-30 min. Aliquots should be prepared with 45 ml in sterile 50 ml tubes (Trypsin will expand upon freezing!) under the clean bench, and stored at -20°C.
Note: The in-use aliquot can be stored at 4°C for several weeks, up to a month.

After opening your clean-bench, start adding all equipment you will use for cell passaging (pipette tips, Pasteur pipettes, RT PBS (1x), new cell culture plates, media and Trypsin once warmed up, 15 ml Falcon). For this, spray everything thoroughly with 70% Ethanol before putting under the hood.
Note: Ensure all materials are sterile and organized before starting.

Inspect Cells

2 Before splitting your cells, inspect your plate.
The media should be translucent (opaque/milky media is a sign of yeast contamination). Under a microscope assess the cell density (for splitting should be around 80%), as well as potential contaminations by higher magnification (bacteria will look like small black dots that move).
Once cleared, move your plate under the hood (NO ETHANOL here!).

Hygiene Note:
- Always wear ethanol-sprayed, clean nitrile gloves. Wear a clean lab coat.
- Make sure that where the plate will go has been cleaned upfront with 70% Ethanol
- Never open the culture plate outside of the sterile hood.

Wash Cells

3 Carefully aspirate old media using a sterile glass Pasteur pipette connected to vacuum.

Gently add 8 ml PBS to one side of the plate side using a serological pipette. Swirl the plate gently and aspirate the PBS with a glass pipette.

Note: Only lift the plate lid with one hand as much as needed to introduce a pipette tip. Always discard all lab ware that was in contact with your culture plate (single use), or that accidentally touched anything else before your cell culture, to minimize risk of contamination.

Detach Cells

4 Add 2ml Trypsin (0.05% or 0.25% depending on your cell type) for a 10 cm plate with a serological pipette. Incubate the plate in the incubator at 37°C for 5min. This step can vary between cell types. Inspect the plate by tilting it to see if cells have detached. Importatn: Do not over-trypsinize to avoid damaging cells.

Resuspend Cells

5 Once cells have detached, under the clean bench, add 6ml of media to neutralize the Trypsin. Resuspend your cells by "washing" down all cells, tilting the plate with one hand. Transfer your resuspended cells to a clean 15 ml falcon tube.

Centrifuge Cells

6 Centrifuge your falcon tube at 1500rpm / 300g for 3-5min, RT.

Plate Cells

7 Remove the supernatend, under the hood, without disrupting the cell pellet.
Resuspend pellet in 5 to 10ml fresh media. Optionally, take 10µl of the resuspended cells for cell counting.
Plate 1×10^6 cells on a new plate containing in total 10ml of media.
Clearly label your plate with the cell type, date, cell number (optional), passage number and your name, before putting the plate in the 37°C incubator (37°C, 5% CO_2 and 95% humidity).

Final Notes

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- Dispose all contaminated labware properly.
- Regularly monitor for mycoplasma contamination.