Basic Cell Culture Maintenance: Splitting Cells

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Abstract

Basic protocol to split Human Embryonic Kidney 293 (HEK293) cells to maintain them throughout the week and move on to cell culture plating with leftover cells for future experiments.

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Before start

Make complete DMEM:

Reagent	*Volume*
DMEM	432.5 mL
FBS	50 mL
Pen/Strep	5 mL
HEPES (1M, pH 7.4)	12.5 mL

Materials

HyClone Classical Liquid Media Dulbeccos Modified Eagles Medium (DMEM) SH3024301 by Fisher Scientific

Gibco™ (Phosphate Buffered Saline) Solution, pH 7.4 (PBS) 10010-049 by Fisher Scientific

Trypsin-EDTA (0.25%), phenol red 25200-056 by Thermofisher

Protocol

Preparation

Step 1.

Confirm that cells are at least 80% confluent by microscopy.

Preparation

Step 2.

Warm complete DMEM in 37°C water bath and thaw trypsin at room temperature.

Preparation

Step 3.

Sterilize the biosafety cabinet with 10% bleach for 20 minutes. Spray down the biosafety cabinet with 70% ethanol and use UV light for 15 minutes as a secondary decontaminant.

Remove Media

Step 4.

Aspirate the media from the flask using a sterile autoclaved glass pipette. **Do not touch the cells with the pipette.**

NOTES

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To avoid touching cells, is best to tilt the flask and gently remove media from a corner.

Wash Cells

Step 5.

Careful not to disturb cells adhered to the wall with the flow, add 5 mL PBS.

Wash Cells

Step 6.

Gently swish PBS over cells to wash off the media by gently rocking it over the cells on the flask wall.

Wash Cells

Step 7.

Quickly, aspirate PBS out. Cells will detach if PBS is left on them for too long.

Trypsinize

Step 8.

Add 3 mL trypsin and gently rock the flask to cover cells.

NOTES

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Trypsin is harmful to the cells. Pay special attention that cells are not in trypsin longer than one minute.

Trypsinize

Step 9.

Incubate flask at 37° for 30 seconds to 1 minute until cells start lifting off.

NOTES

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During incubation, quickly prepare and label a 50 mL falcon tube for the next steps.

Smack!

Step 10.

Remove cells from incubator and quickly, smack! until cells are no longer adhered to the wall.

Neutralize Trypsin

Step 11.

Add 7mL warm complete medium, rinsing multiple times by pipetting up and down, to neutralize the trypsin reaction. Pipette towards cell side of flask walls.

NOTES

David Ellison 13 Mar 2018

Trypsin may destroy your cells if you are not fast enough to neutralize it.

Transfer

Step 12.

Transfer ALL contents/cells to a 50 mL falcon tube.

Spin

Step 13.

Spin down 3,000 rpm for 5 minutes.

Spin

Step 14.

While spinning, clean surfaces with EtOH and label new flasks, noting the +1 passage number and dilution.

Remove Media

Step 15.

Aspirate media from falcon tubes with cells; make sure to not disturb the pellet.

Resuspend Pellet

Step 16.

Add 10 mL media to pellet and pipette *violently* up and down.

Prepare New Flask

Step 17.

Add 8 mL fresh media to new flask, then 2 mL of resuspended cells. (2:10 dilution)
Or 9 mL fresh media, then 1 mL resuspended cells. (1:10 dilution)

Incubate

Step 18.

Gently shuffle, ensure even dispersal, and return the fresh flask to incubator.

Future Experiments

Step 19.

Count and plate cells with leftovers after splitting, then move on to transfection or other experiments tommorow.

Warnings

- Human Embryonic Kidney (HEK293) cells are **biosafety level 2 (BSL-2)** and should be handled according to the CDC's Biosafety in Microbiological and Biomedical Laboratories (BMBL) guidelines. They are considered BSL-2 not because they are inherently hazardous or infectious, but because of their potential to be infected with pathogens and in turn infect their handlers. Due to the impossibility to regularly screen this cell like for every human pathogen, **HEK293 cells should always be handled as potentially infectious**. Other BSL-2 cell lines include those positive for *Legionella pneumophila*, HIV, and other disease-causing pathogens in humans.

- Dispose of ALL waste that comes into contact with cells such as pipettes, gloves, and materials as biohazardous waste.

- Bleach all direct cell waste thoroughly. In our lab, our vacuum line tube empties in to a sealed waste jug with bleach already added to the bottom of it, making up at least 10% of the total volume. This way, aspirated media and cells immediately come into contact with the bleach. Before disposing of glass pipettes, we aspirate a small amount of 10% bleach through to clean both the pipette and tubing, then dispose of the pipettes as biohazardous sharps.