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

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Basic Cell Culture Maintenance: Splitting Cells

Forked from Basic Cell Culture Maintenance: Splitting Cells

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ABSTRACT

Basic protocol to split THP1

MATERIALS

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- HyClone Classical Liquid Media Dulbeccos Modified Eagles Medium (DMEM) **Fisher Scientific Catalog #SH3024301**
- Gibco™ (Phosphate Buffered Saline) Solution, pH 7.4 (PBS) **Fisher Scientific Catalog # 10010-049**
- Trypsin-EDTA (0.25%), phenol red **Thermofisher Catalog #25200-056**

Keywords: cell culture, hek293, cell, maintenance, split, cell splitting, splitting, maintaining cell lines, cell lines, maintain, cells

SAFETY 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.

BEFORE START INSTRUCTIONS

Make complete DMEM:

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

Preparation

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

2 Warm complete RPMI in 37°C water bath.

3 UV light for 30 minutes then spray down the biosafety cabinet with 70% ethanol and use as a secondary decontaminant.

4 Prepare 20 µL of Trypan blue into a 200 µl Eppendorf.

Remove Media

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

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

Transfer

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

Spin

7 Spin down 130 rcf for 7 minutes. 00:07:00 130 x g, 37°C 7m

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

Remove Media

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

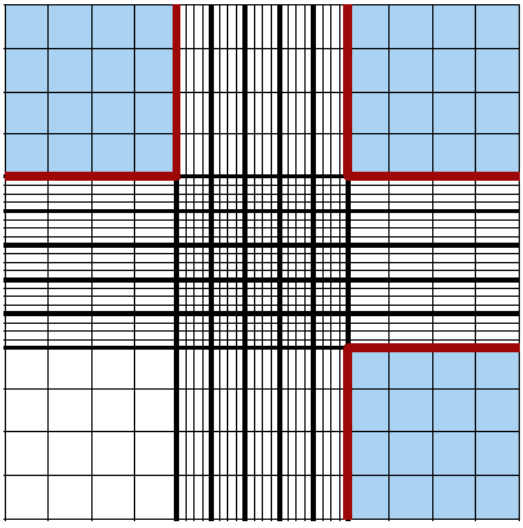
Resuspend Pellet

10 Add 5 mL media to pellet and pipette *violently* up and down.

Count the cells

11 Pipette 20 µL and add them to the Trypan blue

12 Count the cells using a Neubauer counting chamber



$$\mathcal{N} = \frac{n_{cells}}{n_{squares}} \cdot \mathcal{D} \cdot \mathcal{V}_{tot} \cdot 10000$$

13 Calculate the required amount of medium to keep a concentration of [M] 100 kC/ml

Prepare New Flask

14 Add the required amount of medium.

15 Distribute in flasks, no more than 50 mL per flask.

Incubate

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