Apr 24, 2018

Basic Cell Culture Maintenance: Plating Cells

DOI
dx.doi.org/10.17504/protocols.io.pqgdmtw

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DOI: https://dx.doi.org/10.17504/protocols.io.pqgdmtw

Protocol Citation: Britt DK Gratreak 2018. Basic Cell Culture Maintenance: Plating Cells. **protocols.io https://dx.doi.org/10.17504/protocols.io.pqgdmtw**

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

Created: April 24, 2018

Last Modified: April 24, 2018

Protocol Integer ID: 11752

Keywords: HEK293, cell culture, cells, plate, plating, 12 well, 12 well plate, seed, seeding, ellison lab, basic cell culture maintenance, plating cell, human embryonic kidney, cell, kidney, well plate

Abstract

Basic protocol to plate Human Embryonic Kidney 293 (HEK293) cells in to 12 well plates. This protocol can be modified easily to plate in different volumes and concentrations.

Materials

MATERIALS

- HyClone Classical Liquid Media Dulbeccos Modified Eagles Medium (DMEM) **Fisher Scientific Catalog #**SH3024301
- Hausser Scientific Bright-Line™ Counting Chamber **Fisher Scientific Catalog #**02-671-51B
- Falcon™ Polystyrene Microplate (12 well) **Fisher Scientific Catalog #**08-772-29

STEP MATERIALS

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Troubleshooting

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

Make complete DMEM:

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

Split Cells

1 Split cells following the **Basic Cell Culture Maintenance: Splitting Cells** protocol. Cells leftover from split flasks will be used to count and plate cells.

Counting Cells

2 Add 75 µL media and 25 µL leftover cells into a 1.5 mL microcentrifuge tube. (1:4 dilution)

Note

To avoid contamination, it is best practice to aliquot a small amount of the media for this purpose by using a sterile serological pipette. Always avoid using a micropipette to directly make contact with the media stock.

3 Mix thoroughly by flicking.

4 Load 10 µL into the hemacytometer and view under microscope.

Hausser Scientific Bright-Line™ Counting Chamber **Fisher Scientific Catalog #**02-671-51B

Calculating Concentration

5 Count cells per quadrant. Average them. Multiply by 10,000 and the dilution factor (4).

Note

For example, if you counted 66, 65, 58, and 70 cells in their individual quadrants, they would average to **64.75 cells per quadrant**. Multiply this by **10,000** and then by **4**. This will lead to determining a concentration of **2,590,000 cells per mL** in the leftover cells from splitting.

Creating a Master Mix

6 Calculate amount of cells + new media needed to create a master mix using C_1V_1, in which concentration is cells per mL/cells per well. (one well = 1 mL) Add 3 more wells of volume than needed to calculations to account for errors.

Note

Using the example previously, 2,590,000 cells per mL is the concentration of cells that are leftover from splitting. The amount of cells to plate depends on the purpose for the cells and the rate of growth of the cell line. Typically, **Lipofectamine 2000 transfection** with HEK293 cells works best with plating 500,000 cells per well. For a 12 well plate, add 3 extra wells to account for any pipetting error/interfering bubbles.

The equation to solve for would be:

'HAVE' vs 'WANT'
C1V1 = C2V2
2,590,000 cells/mL * (X mL) = 500,000 cells/mL * (15 mL)
X mL = (500,000 cells/mL * (15 mL)) / 2,590,000 cells/mL

X = 2.9 mL cells
15 - 2.9 mL = 12.1 mL media

Thus, your master mix would consist of:
2.9 mL cells
+ 12.1 mL media
= 15 mL total.

7 Create master mix in 50 mL falcon tube. Invert master mix several times just before plating to ensure it is evenly mixed.

Plating

8 Label plates with strain, date, and initials.

9 Add 1 mL master mix to each well.

Incubate

10 Gently mix plates with gentle shaking. Return the cells to the incubator at 37°C and 5% CO2.