

Basic Cell Culture Maintenance: Plating Cells

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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.

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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](#)

Hausser Scientific Bright-Line™ Counting Chamber [02-671-51B](#) by [Fisher Scientific](#)

Falcon™ Polystyrene Microplate (12 well) [08-772-29](#) by [Fisher Scientific](#)

Protocol

Split Cells

Step 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

Step 2.

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

NOTES

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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.

Step 3.

Mix thoroughly by flicking.

Step 4.

Load 10 μ L into the hemacytometer and view under microscope.

REAGENTS

Hausser Scientific Bright-Line™ Counting Chamber [02-671-51B](#) by [Fisher Scientific](#)

Calculating Concentration

Step 5.

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

NOTES

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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

Step 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.

NOTES

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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'

$$C1V1 = C2V2$$

$$2,590,000 \text{ cells/mL} * (X \text{ mL}) = 500,000 \text{ cells/mL} * (15 \text{ mL})$$

$$X \text{ mL} = (500,000 \text{ cells/mL} * (15 \text{ mL})) / 2,590,000 \text{ cells/mL}$$

$$X = 2.9 \text{ mL cells}$$

$$15 - 2.9 \text{ mL} = 12.1 \text{ mL media}$$

Thus, your master mix would consist of:

2.9 mL cells

+ 12.1 mL media

= 15 mL total.

Step 7.

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

Plating

Step 8.

Label plates with strain, date, and initials.

Step 9.

Add 1 mL master mix to each well.

Incubate

Step 10.

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

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 line 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.