Routine Cell Culture

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Abstract

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Protocol

Prepare complete media

Step 1.
Most cell lines I work with are cultured in standard DMEM + 10% FBS + 1% Anti/Anti. Prepare this reagent by mixing 1 bottle (500 mL) of DMEM with 50 mL of FBS and 5 mL of Anti/Anti. Shake to mix. This reagent is now ready to use and can be stored in the fridge for several weeks/months.

Prepare for cell culture

Step 2.
Remove the appropriate bottles of growth media and trypsin-EDTA from the fridge and pre-warm them in a water bath for 10-15 minutes. Wipe down each bottle with 70% ethanol then move them to the tissue culture hood. Clean a bottle of sterile PBS (stored at room temperature) and move it to the hood. Move the appropriate tissue culture plastics into the hood (e.g. 10 cm plates or 6-well plates).

Inspect Cells

Step 3.
Remove the appropriate plate of cells from the incubator and visually inspect the cells to ensure that they are at the appropriate confluence. I typically passage my cells at approximately 75% confluence. If cells are overconfluent, you should throw the cells away and thaw a new vial. At high power magnification, inspect the plate of cells to ensure that there is no obvious contamination. If the plate of cells appears okay, move it to the cell culture hood.

Rinse with PBS

Step 4.
Add PBS to the plate, swirl, and remove. Add the PBS to the wall of the plate, and take care to ensure that the cells are not dry for too long.

AMOUNT

10 ml Additional info:

REAGENTS

PBS by Contributed by users

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

Trypsinize

Step 5.

Add trypsin, then incubate plates until the cells detach. I typically incubate the plates at room temperature for approximately 2 minutes, but some cell lines may required trypsinization at 37C or for a longer period of time.

AMOUNT

2 ml Additional info: This is the appropriate volume for a 10-cm plate.

TEMPERATURE

23 °C Additional info:

READENTS

Trypsin-EDTA (0.25%) 500 mL
7901 by Stemcell Technologies

Inspect Cells

Step 6.

Inspect cells with the naked eye or under the microscope. If cells are detached from the plate, proceed to the next step. If cells are stuck to the plate still, tapping the plate may help remove them. Otherwise, simply incubate cells for longer.

Resuspend Cells

Step 7.

Add complete media to the plate and pipete up and down to break up clumps of cells.

AMOUNT

5 ml Additional info: This amount is appropriate for a 10-cm plate

Seed cells

Step 8.

Add 10 mL of complete media to a new 10-cm cell culture plate. Add an appropriate amount of cell suspension to this plate. I typically add 1 mL to the new plate for most cell lines. The remaining cell suspension can either be used for experiments or thrown away.