



Jun 15, 2018

Routine Cell Culture

DOI

dx.doi.org/10.17504/protocols.io.q2jdycn

Sean Misek¹

¹Michigan State University

Neubig_Lab



Sean Misek

OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.q2jdycn

Protocol Citation: Sean Misek 2018. Routine Cell Culture. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.q2jdycn>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: June 15, 2018


Last Modified: June 15, 2018

Protocol Integer ID: 13099

Materials


STEP MATERIALS

 PBS

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

 Trypsin-EDTA (0.25%) 500 mL **STEMCELL Technologies Inc. Catalog #7901**

 PBS


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

 Trypsin-EDTA (0.25%) 500 mL **STEMCELL Technologies Inc. Catalog #7901**

Protocol materials


 Trypsin-EDTA (0.25%) 500 mL **STEMCELL Technologies Inc. Catalog #7901**

 PBS


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

 Trypsin-EDTA (0.25%) 500 mL **STEMCELL Technologies Inc. Catalog #7901**

 PBS

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

 PBS

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

 Trypsin-EDTA (0.25%) 500 mL **STEMCELL Technologies Inc. Catalog #7901**



Prepare complete media

- 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

- 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


- 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

- 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.

 PBS


 10 mL

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

Trypsinize

- 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 require trypsinization at 37C or for a longer period of time.

 Trypsin-EDTA (0.25%) 500 mL **STEMCELL Technologies Inc. Catalog #7901**

 2 mL This is the appropriate volume for a 10-cm plate.



23 °C

00:02:00

Inspect Cells

- 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

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

5 mL This amount is appropriate for a 10-cm plate

Seed cells

- 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.