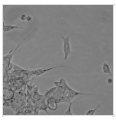

Feb 26, 2019

Working

Mammalian Cell Culture: Refreshing Media

Forked from Mammalian Cell Culture: Refreshing Media

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

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ABSTRACT

This protocol explains how to refresh the media of cultured mammalian cells grown in a tissue culture flask.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Gloves must be worn at all times. Perform all tasks within biosafety cabinet.

MATERIALS TEXT

- Gloves
- Cultured T-75 flask
- or Cultured T-25 flask
- Serological pipette with tips
- Dulbecco's Phosphate Buffered Saline (DPBS)
- Cell culture media (e.g. DMEM:F12, EMEM, etc.)

BEFORE STARTING

Warm cell culture media and DPBS in 37C water bath. UV serological pipette tips and waste beaker.

Assessing Culture Health

1 Assess cell health under light microscope before beginning.

Refreshing media

2 Using a serological pipette, remove old media from flask and transfer to waste beaker.

To avoid contamination, avoid touching the pipette tip to anything. Holding the flask such that the lid is pointed up will make it easier to remove all the liquid.

3 Wash cells by pipetting 1 ml warmed DPBS into the flask.

4 Remove DPBS and dispose into waste beaker.

5 Repeat the above two steps for a total of 2 washes.

6 Pipette 4 ml warmed cell culture media into flask.

Incubate

7 Spray flask generously with 70% Ethanol solution before placing in CO_2 incubator.