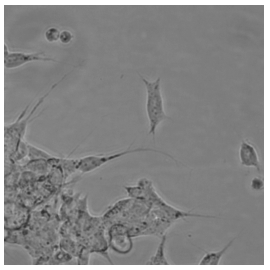
Jun 05, 2019 Version 2

Mammalian Cell Culture: Refreshing Media V.2



Version 1 is forked from Mammalian Cell Culture: Refreshing Media

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

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Abstract

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

Guidelines

- Gloves must be worn at all times.
- Perform all tasks within biosafety cabinet.
- Anything entering biosafety cabinet must be generously sprayed with 70% ethanol (even you).
- When finished, wipe biosafety cabinet with 70% ethanol, and UV for at least 15 minutes.

Materials

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

Before start

- Warm cell culture media and DPBS in 37 °C water bath.
- Wash waste beaker with soap and warm water, then dry with paper towel.
- Expose serological pipet tips and waste beaker to UV for at least 00:15:00 .

Assessing Culture Health

1 Assess cell health under light microscope before beginning.

Refreshing media

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

Note

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 4 mL warmed DPBS into the flask. [1 mL for T-25 flask]

4 Remove DPBS and dispose into waste beaker.

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

6 Pipette 8 mL - 12 mL warmed cell culture media into flask. [4 mL for T-25 flask].

Incubate

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