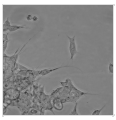

# Mammalian Cell Culture: Refreshing Media

Kenneth Schackart[1], Kattika Kaarj[1]

[1]University of Arizona

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481b Laboratory

Kenneth Schackart

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

Labcoat and gloves must be worn at all times.

MATERIALS TEXT

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

## Assessing Culture Health

1 Look at cells cultured in T-75 flask using the light microscope.

NOTE

Separate protocol for microscope use provided at microscope station and online.

2 Take image of the cells.

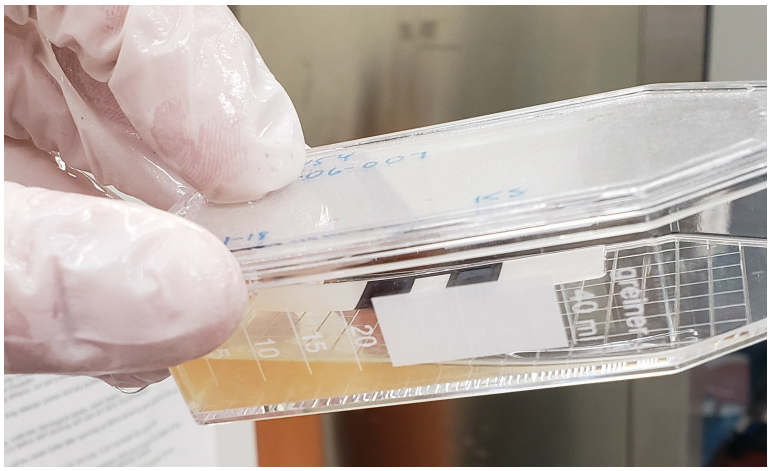
**Iclude this image in the Results section of the lab report.**

**Include an assessment of culture health based on cell morphology in Discussion section.**

NOTE

If you have been feeding too infrequently, you may see signs of poor health, such as cells rounding or looking less attached to the surface. You may also see the media turn yellowish orange, which is evidence of a pH change (most cell media have phenol red, a pH indicator)

This is a good time to check for contamination. Sometimes, contamination is immediately evident if culture media has become turbid. Or you may see bacteria moving when viewing under the microscope.



Contamination evidenced by media turbidity and color.

## Refreshing media

3 Using a serological pipette, remove old media from flask. Dispose of media in waste beaker. Dispose of pipette tip by sliding it back into its wrapper, and disposing in sharps box.

> 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.

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

5 Remove DPBS and dispose into waste beaker.

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

7 Pipette 4 ml warmed cell culture media into flask.

## Incubate

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

> NOTE
> This will be performed by T.A.