MUSC
MEDICAL UNIVERSITY
of SOUTH CAROLINA

Ferreira Laboratory
86 Jonathan Lucas St
HO606 (Lab)
MSC635/HO612h (Office)
Charleston SC 29425-5090
Tel 843 792 0614
www.ferreiralab.com

Making Cell Culture Media/Sterile Technique Protocol

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Materials:

Product	Amount Used*	Supplier	Cat. No.
DMEM	500 ml	ThermoFisher	11960069
**RPMI1640	500 ml	ThermoFisher	21870092
FBS	50 ml	ThermoFisher	A3840202
HEPES	5 ml	ThermoFisher	15630130
Pen-strep (P/S)	5 ml	ThermoFisher	15140163
Nonessential amino acids (NEAA)	5 ml	ThermoFisher	11140050
GlutaMAX (gMAX)	5 ml	ThermoFisher	35050079
Sodium pyruvate (Pyr)	5 ml	ThermoFisher	11360070

*makes 1 bottle of media (~575 ml)
**DMEM and RPMI are interchangeable basal media

VWR 5 ml serological pipettes (Cat No. 89130-908)
VWR 25 ml serological pipettes (Cat. No. 89130-900)
Drummond Original Pipet-Aid Pipette Controller (VWR, Cat. No. 53498-105)
Millipore Stericup 500 ml Durapore 0.45µm PVDF (Cat. No. SCHVU05RE)

Reagents Setup:

1. Make sure FBS and Penicillin-Streptomycin (pen-strep) are taken out ahead of time if they are frozen to allow time for thawing in a water bath at 37°C

    a. pen-strep/FBS can't stay in the fridge for too long or they go bad so if there is leftover reagent, make 5 ml aliquots in 15 ml falcon tubes and place in the -20 freezer
    b. FBS cannot be left out of the fridge overnight or it will go bad
2. Order of addition of the reagents does not matter so pick your first reagent and place it on the same side as your dominant hand. Then place the DMEM on the other side. This will allow you to move quickly between the two —> prevents dripping/contamination of the tissue culture (TC) hood
3. Make sure there is only one reagent in front of you at a time, move all others to the side for later use
4. When using a reagent, be sure to write the latest date of use on the bottle so as to keep track of how much we've used over a certain period of time - that way we can tailor the ordering process to our experiment protocols

General Sterile Technique Reminders:

1. Wipe down the hood with 70% ethanol (EtOH) both before and after you finish working in that space
2. Do not pipette in front of tubes, only pipette in front of a clear space
3. Remember not to work over the tubes too much
4. Keep hands/materials away from the edge of the hood, try to work closer to the back to most effectively prevent contamination
5. Before the filtration step in making the media, there is less of an emphasis on sterile technique since the filter itself sterilizes the media. However, once the filtration step is complete, sterile technique needs to be exercised with greater caution
6. When pipetting, try to make smooth, continuous movements to prevent dripping/contamination.
7. Do not insert air while pipetting, to avoid bubbles. Do this by not pipetting all the way up or all the way down. Always have the tip of the pipette submerged in liquid
8. Keep your caps loose to make it easier to remove and replace them without breaking sterile technique while pipetting
9. In the event a significant amount of a substance spills inside the hood, first use water to wipe it up and then use 70% EtOH

Procedures:

1. Wipe down the hood and arrange materials as described above
2. Unscrew the caps to all of the reagents, but still keeping them loosely on - be sure to do this before the next step
    a. If you forget to unscrew the caps before grabbing and unwrapping a sterile serological pipette: throw away that pipette, open the caps, and grab a new serological pipette (better to waste the pipette than to have contamination affect the results of an entire experiment)

3. Use a sterile 25 ml serological pipette and the Drummond Pipet Aid Controller to add 50 ml of FBS to the 500 ml of DMEM, using sterile technique
    a. When transferring 50 ml of FBS to the DMEM, quickly remove the cap on the DMEM, add the FBS, and immediately re-cap on the media (keep the media covered as often as possible to prevent contamination)
    b. The FBS we have currently is already packaged as 50 ml aliquots so pipetting is not necessary for us - directly pour the FBS into the media without touching the rim
4. Use a sterile 5 ml serological pipette to add 5 ml of *HEPES to the media, remembering to immediately re-cap the media once the volume has been added —> repeat this procedure for the 4 remaining reagents (5 ml Pyr, pen-strep, NEAA, gMAX), using a new sterile 5 ml serological pipette for each successive reagent
    a. *order of addition of reagents has no effect)
5. Once all reagents have been added, tightly close the cap on the media and invert the container twice to mix
6. Filtration
    a. Open a sterile 500 ml Stericup filtration system and place the cap packaged separately to the side - do not remove its packaging yet
    b. Check to make sure the bottom portion and the top portion of the filter are screwed on as tight as possible
    c. Connect the filter to the vacuum before pouring in your media
    d. Turn on the vacuum by moving the white lever inside the hood from a vertical position to a horizontal position. There should now be airflow
    e. Pour in the media and wait until it collects in its entirety in the bottom portion of the filter
    f. Turn the vacuum off by returning it to the vertical position and carefully disconnect the filter from the vacuum (the piece connecting the filter to the vacuum that comes off once filtration is complete can then be discarded in a biohazard bin)
    g. Before the next step, remove the top portion of the filter by unscrewing it and then dispose of it. You should be left with only the bottom portion of the filter containing your freshly sterilized media and the pre-packaged cap set aside earlier
    h. Immediately, remove the sterile cap from its packaging without touching the inside of the cap and screw it tightly on the bottom portion of the filter containing the media (remember to be extra conscious of your sterile technique during these last few steps because the filtration step is now complete)
7. Label the bottle with the name of the media (DMEM10 in this example), components, initials, and date (also label the cap with the name of the media and initials so it's easier to find in the fridge)
8. Immediately **store your media in the refrigerator
    a. **media is both light and temperature sensitive so it must go in the fridge)

9. Wipe down the hood again with 70% EtOH and turn on the UV light for ~3 mins (do not leave it on overnight or for long periods of time)
10. Put all of your reagents away:
    a. Leftover Pen-strep can't stay in the fridge —> 5 ml aliquots —> -20C freezer
    b. Leftover FBS can't stay in the fridge —> 50 ml aliquots —> -20C freezer (won't be the case for us for a while because we have FBS in the form of 50 ml aliquots currently)
    c. All other reagents can go back in the fridge

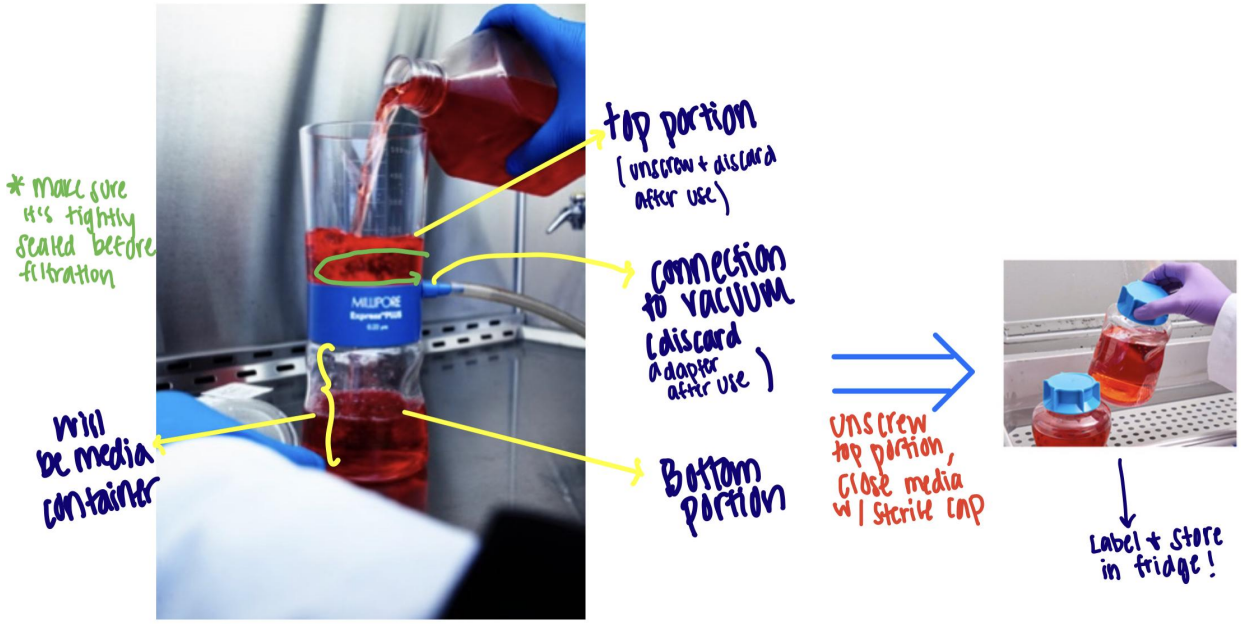

Figure 1. Workflow to Make and Sterilize Cell Culture Media

Supplemental Information:

- We must add all of these additional components to the basal DMEM media because the cells need them to grow, but the media cannot come pre-made with all of the components already added since they degrade easily. If it were pre-made, the reagents would become unstable and create a toxic environment for the cells
    - GlutaMAX is a stable version of glutamine (necessary because when glutamine becomes unstable its NH_2 group converts to NH_3 —> ammonia, which is toxic to cells)
- Pen-strep prevents contamination
- HEPES acts as a buffer
- RPMI1640 can be used in place of DMEM as a basal medium. Different cell types prefer different kinds of media. For example, DMEM10 is used to culture HEK293T cells, whereas RPMI is used to culture Jurkat cells and primary human T cells and Tregs

- The RPMI used in this laboratory does not contain glutamine
- The filter sterilizes the media because it has pores that are smaller than bacteria and other microbes so the contaminants never make it past the filter and are trapped in the top portion, while the media is freshly sterilized in the bottom portion of the filter
- The use of the UV light at the end of your experiment will be particularly important when we work with virus