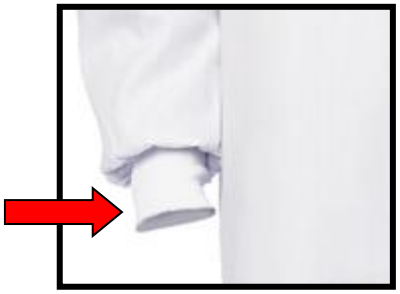
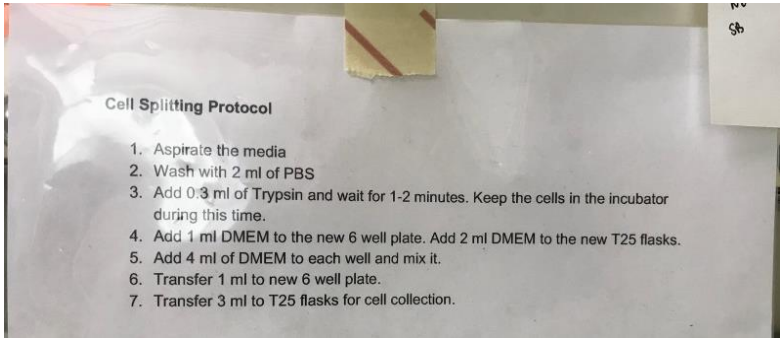
BSL-2 ENHANCED WORK PRACTICES FOR CELL CULTURE

Personal Protective Equipment		
Wear a buttoned-up lab coat with cuffed sleeves.	Have dedicated lab coats for cell culture work. *Place hooks in the tissue culture room.*	Use disposable sleeve covers to minimize contamination.
		
Double gloves are recommended for BSL-2 experiments.	Disinfect or remove outer pair of gloves prior to exiting the biosafety cabinet.	Consider the use of gloves with extended cuffs to ensure your gloves extend over lab coat sleeves.
		

Setup of Biosafety Cabinet (BSC)

Place the protocol with step-by-step instructions on the outside of the BSC. (Never place it inside the BSC)



Note: *Avoid having unnecessary items that you do not need for your experiment inside the BSC; this helps you with the proper decontamination process of the BSC.*

Place disinfectant spray bottles inside the BSC.

This includes a list of things you need to bring inside the BSC.

Cell splitting

____ 5ml pipet

____ Pipetman

____ 1000ul Pipet

____ 1000ul pipet tips

____ T25 Filter flasks

____ 6well plate

____ PBS

____ Trypsin

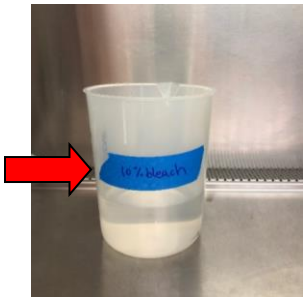
____ DMEM

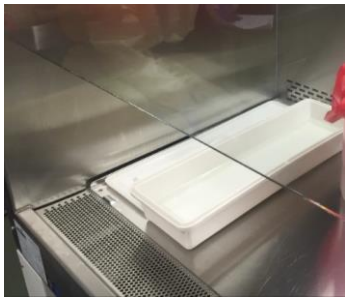
Notes:

- *Leave the bottles inside the BSC for the remaining time until your experiment is done.*
- *This ensures that you can disinfect your items prior to removal without removing hands out of the BSC.*
- *It also prevents disruption of the protective front air barrier and spread of contamination by gloves outside the BSC.*

Setup of Biosafety Cabinet (BSC)-Continued

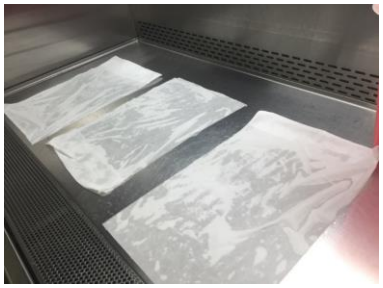
Set up containment to collect waste inside the BSC.
Set up a beaker or horizontal tray containing disinfectant or serological pipets.
Label the container with the name of disinfectant.



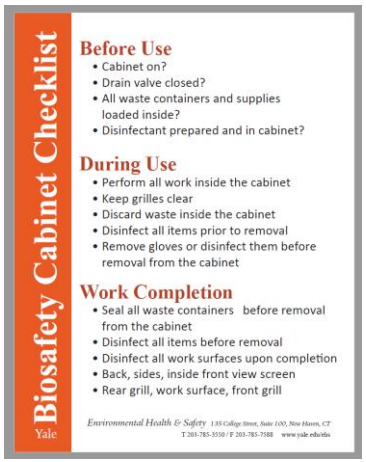


Set up a biohazard bag for solid waste.

If you have large items, consider placing disinfectant-soaked paper towels down in work area which helps to disinfect the bottom of these items.

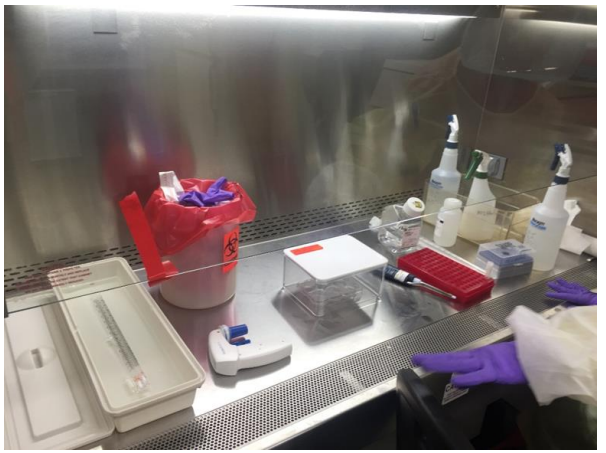


Follow the instructions on the BSC checklist before starting your work.



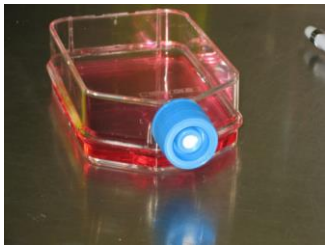
Setup of Biosafety Cabinet (BSC)-Continued

- Here is an example of an appropriate set up of the BSC with a "clean" vs "dirty" area.
- Take time to organize the BSC prior to starting work.
- Notice the location of the waste collection containers, cleaning supplies and disinfectant bottles in the picture.



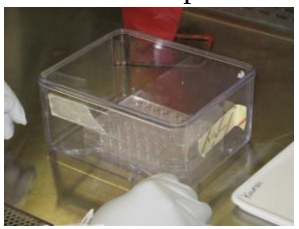
Engineering Controls (Safety Equipment and Supplies)

Sealable filtered flasks



Sealable secondary containers for transport

e.g., for transport out of lab



e.g., for transport of tissue culture plates from BSC to incubator

Sealable aerosol-tight secondary containers for centrifuge

Note: All major centrifuge manufacturers have designed secondary containers for biohazardous materials available for purchase

Engineering Controls (Safety Equipment and Supplies)-Continued

Make sure to use tubes as primary containers that are also sealable (e.g., screw cap tubes with O-ring).

Use filtered serological pipets and filtered pipette tips.

Avoid the use of sharp items (e.g., glass Pasteur pipettes, needles and syringes) for cell culture experiments. *If possible, use the plastic alternatives and/or safe sharp devices.*

e.g., plastic aspirating pipets

Note: If you must use a sharp such as a needle and/or syringe, use a blunt end needle for aspiration of liquid. Please notify EHS for an evaluation of sharps use in cell culture experiments before initiation.

Follow Safe Work Practices

- Work slowly to minimize the amount of aerosols generated.
- Minimize airflow disruption by moving hands in and out of the BSC less often.
- Never block the front or rear grills of the BSC.

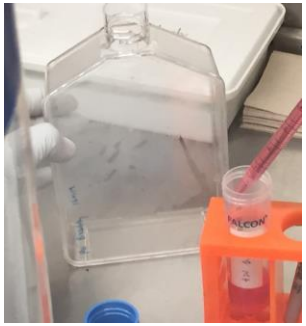
Rear airflow grill

Front airflow grill

Follow Safe Work Practices-Continued

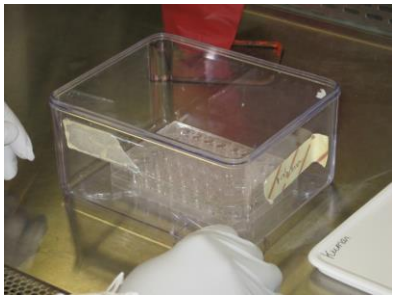
Pipetting can generate aerosols. To minimize aerosols:

- Release liquids against the side wall of the flask/tube.
- Re-suspend cells by carefully pipetting up and down.
    - Do not release all liquid from pipet. Never forcibly expel liquids from pipettes.
- Never dispense liquid from a height as this creates more aerosols.



To reduce potential spread of contamination outside the BSC:

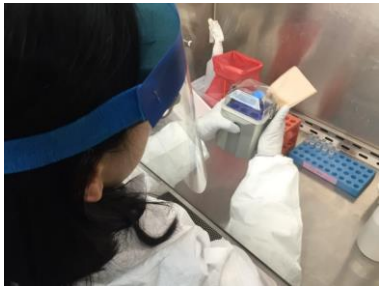
- Disinfect exterior of tubes and tissues culture plates and then load into secondary container inside the BSC.
- Disinfect exterior of secondary container prior to removal from BSC.



- Load and unload centrifuge rotors and/or safety buckets within the BSC.
- Never load secondary container outside of the BSC.

Follow Safe Work Practices-Continued

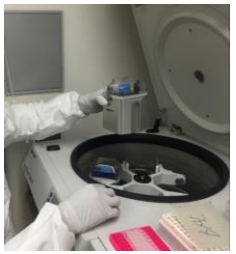
- Disinfect safety buckets prior to removal from BSC.



Transport to and from the BSC

- Use secondary containers for safe transport of tissue culture plates to the incubator.

- Use safety buckets for safe transport of centrifugation tubes to the centrifuge.



- Open transport container inside incubator.

Collecting Waste Inside the BSC

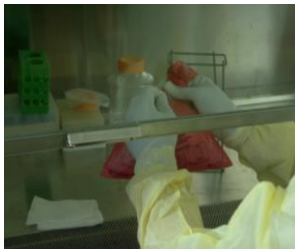
Use a beaker or tray containing fresh* disinfectant for collection and disinfection of pipets

- Draw disinfectant up inside serological pipet and allow to run down into beaker
- Spray the top of the pipet in the beaker before removal from BSC

- Allow at least a 30-minute contact time for full decontamination.

*Preferably prepare fresh 10% bleach solution in water daily. At minimum, you must change the disinfectant solution weekly to ensure its effectiveness.

- Use small biohazard bags for dry waste (pipet wrappers, used gloves, used paper towels) for items that cannot puncture a bag.
- Do not use this waste bag for items that can puncture a bag (e.g., pipet tips).
- Seal biohazard bag with dry waste prior to decontamination of the BSC.



- Spray or wipe down the outside of the biohazard bag wth disinfectant.

Collecting Waste Inside the BSC-Continued

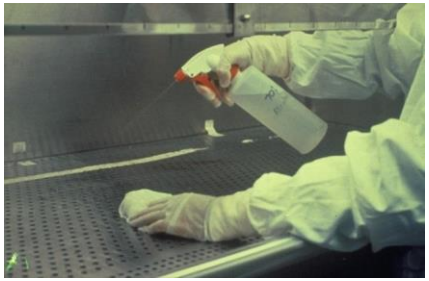
- For items that <u>can</u> puncture a biohazard dry waste bag, use an empty 500ml media bottle to collect pipette tips in 10% household bleach.

- Allow sufficient contact time (based on your biohazards) prior to removal of items from BSC.

Disinfection of BSC

- Disinfect the BSC with 1-10% household bleach in water, followed with 70% Ethanol to remove any bleach residues, which corrode the BSC.
- Disinfect all surfaces (back, sides, inside front view screen, grilles and work surface) by spraying disinfectant.

Note: Alternative disinfectants for decontamination of the biosafety cabinet may be used. Consult EHS to verify the effectiveness of new disinfectants.



Disinfection of BSC-Continued

- Use disinfectant-soaked paper towels to spread the disinfectant in order to get the best surface coverage.

- Consider using a metal swiffer or extension cleaning tool for difficult to reach spots.

After Your Experiment

- Remove your PPE before leaving the tissue culture room.
  - First, remove your outer gloves, then your lab coat, followed by your inner gloves.

- Wash your hands with soap and water after removing your PPE.