

CHILDREN'S HOSPITAL LOS ANGELES STEM CELL CORE PROCEDURES FOR HANDLING CELLS AND OTHER BIOLOGICAL MATERIALS

General regulations

1. All biological materials must be handled in the biosafety cabinets at all times. Fixed cells may be handled in the main lab.
2. A lab coat and gloves must be worn at all times when working with biological materials.
3. Ensure all dishes and flasks are labeled clearly with the investigators name and the name of the cell line / tissue.
4. Do not insert serological or glass pipettes that have come in contact with biological material into sterile medium bottles or containers.
5. Do not cross contaminate cell lines by inserting serological or glass pipettes into multiple dishes containing different cell types.
6. Remove culture medium that splashes into the lids and 'necks' of culture flasks and dishes by aspiration.
7. Do not work with multiple dishes simultaneously. Do not remove the lids from multiple dishes at the same time, as this may lead to cell line cross contamination.
8. Please open and seal packages containing 35mm dishes in the biosafety cabinet.
9. Live cells must be sealed with parafilm, wrapped in a plastic bag and placed in an airtight plastic container for transport – please consult with USC Stem Cell Core staff before transporting live cells.
10. Please minimize movement of live cells around the lab, keep live cells in cell culture rooms.
11. Do not bring outside cell lines into the Core without first consulting with the Core staff for instructions.
12. Do not work with live tissues, human or otherwise at the Core without first consulting with the Stem Cell Core staff.

Medium and solutions

1. Filter units should be checked for cracks and faulty membranes prior to the production of medium.
2. All media bottles and tubes must be cleaned thoroughly with 70% alcohol before placing into the biosafety cabinet.
3. The caps to media bottles and tubes must be sealed with parafilm during storage and warming.

Incubator etiquette

1. Incubators should be kept clean and tidy at all times.
 2. Please store all cells in one location in the same incubator
 3. Please open and close incubator doors carefully to prevent disturbing the cells they contain.
 4. Ensure all spills are cleaned using 70% alcohol.
 5. Work 3 inches inside the clean bench at all times to ensure protection from the outside atmosphere. Ensure cells and pipettes do not accidentally leave the cabinet during cell culture procedures.
 6. Do not leave the lids of dishes open for any longer than is absolutely necessary to complete your work.
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Clean bench regulations

1. Keep a safe distance of at least 2 (feet) when walking behind students working at the clean bench.
2. Hair should be tied back and wear the appropriate protective equipment when working at the clean bench.
3. Work 3 inches inside the clean bench at all times to ensure protection from the outside atmosphere
4. Do not leave the lids on dishes open for any longer than is absolutely necessary to complete your work.
5. Ensure all spills are cleaned using 70% alcohol
6. Ensure the clean bench is left tidy and sterile for the next person

Biosafety cabinet regulations

1. The biosafety cabinets and vacuum lines must be sterilized using 70% alcohol prior to use and cleaned regularly.
2. All personnel are required to tie back their hair and wear the appropriate protective equipment when working in the biosafety cabinet.
3. All personnel are required to tie back their hair and wear the appropriate protective equipment when working in the biosafety cabinet.
4. Work 3 inches inside the clean bench at all times to ensure protection from the outside atmosphere. Ensure cells and pipettes do not accidentally leave the cabinet during cell culture procedures.
5. Do not leave the lids of dishes open for any longer than is absolutely necessary to complete your work.
6. Do not place equipment onto the grill at the front of the cabinet. This must be kept clear at all times.
7. Equipment and media bottles must be sterilized using 70% alcohol before placing into the biosafety cabinet. Supplies such as serological pipettes do not need to be cleaned, providing they have been kept covered in a plastic bag.
8. Do not overcrowd the cabinet with equipment or supplies.
9. Do not place equipment along the back wall of the cabinet. The back grill must be kept clear at all times. Please locate equipment to the sides of the cabinet.
10. Do not rest serological or glass pipettes in the cabinet between procedures. If you need to put down the pipette aid or vacuum line throw away the pipette attached to it.
11. Avoid placing caps and lids onto the surface of the cabinet.
12. Ensure all spills are cleaned using 70% alcohol.
13. Ensure the clean bench is left tidy and sterile for the next student.
14. All vacuum lines must be filtered to prevent the spread of infectious aerosols
15. Ensure the biosafety cabinet is sterilized using UV according to posted instructions.

Disposing of cells and biological material

Please see waste disposal SOP for details.

In the event of a contamination

DO NOT RETURN CONTAMINATED DISHES TO THE INCUBATOR. They must be sterilized and discarded. Please contact a member of the USC Stem Cell Core staff for assistance if your cells acquire an infection.

