

**STANDARD OPERATING PROCEDURE FOR SCAC
(June 2022)**

GENERAL

1. Do not use SCAC Equipment without proper training.
2. Handle SCAC property with care.
3. Clean up after each use.
4. Return the equipment to its original location (if applicable).
5. Let the staff know if you encounter a problem.

SCAC STAFF RESPONSIBILITIES:

1. Maintaining stocks of plasticware, available to users at cost, in SRT303A. Complete list is printed and posted in SRT 303A and SKU's list is on CoreConnect.
2. Maintaining stocks of media and other liquid items, available to users at cost, in SRT 306B or 330B.
3. Providing autoclaved Pasteur pipets in metal boxes located by the flammable cabinet in SRT307C.
4. Providing 4-liter bottles of 70% ethanol located in the flammable cabinet in SRT307C.
5. Providing 100% bleach bottles located in the cabinet under the sink in SRT307C and cabinet in the 306A.
6. Providing autoclaved water bottles to refill incubators.
7. Ordering replacement CO2 and LN2 tanks.
8. Providing floor sticky pads in front of or inside SRT305A, 305B, 306A, 307C doors for reducing dirt entering labs on shoes and minimizing chance of contamination.
9. Providing light bulbs for the microscopes.
10. Providing linens for defrosting freezers.
11. Monitoring of Isensix alarms that are installed in all the -20°C, -80°C, and LN2 freezers. If we notice temperature fluctuations that persist, we will notify the appropriate user/lab by text or email.
12. Overseeing of equipment repair when needed.

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USER RESPONSIBILITIES:

1. Notifying SCAC Manager, Narine Harutyunyan, when your lab has a new member joining to enable badge access to the rooms.
2. Ensuring all users have the appropriate lab safety trainings. Please contact the Lab Safety Office at labsafety@chla.usc.edu for further details.
3. Maintaining cleanliness in the rooms/labs.
4. Signing out plastic items taken from Storage Room-303A. Complete list is printed and posted. Please use the computer and enter your Order on CoreConnect. These items are then charged on the next monthly bill in which PI's receive detailed charges. Note: SCAC is charging all the items at cost.
5. Writing on the whiteboard (on the inside of the 303A door) any item in which user opens the second to last box, so we can order it in a timely manner.
6. Signing out of media and liquid items taken using the sheet on the SRT 306B refrigerator door. Note: SCAC is charging all the items at cost.
7. Sharing of all equipment including incubators, refrigerator, freezer, microscope, centrifuge, as well as common spaces (such as bench space and cabinets) when there is more than one lab using same room.
8. Providing lab coats for each person using the Core. For information on how to order lab coats please email Lab Safety Office at labsafety@chla.usc.edu.
9. Note that dirty lab coats can be dropped off at the provided linen hamper located next to the SRT 3rd floor elevator.
10. Refilling the incubators with water when alarm sounds. Autoclaved water is supplied in 307C.
11. Monitoring incubator CO2 levels daily and replacing the CO2 tank if the level is low or empty. A sticky note with replacement date should be posted on the tank. Please ask Andrew if you have questions or need training on tank changing. Also, notify Andrew to reorder tank.
12. Returning empty metal Pasteur pipet boxes to the flammable cabinet in SRT307C for refill.
13. Refilling 70% ethanol spray bottles at the hoods being used.
14. Returning empty 4-liter bottles of 70% ethanol into the flammable cabinet in SRT307C for refill.

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15. Emptying the vacuum flask under each hood before overflowing using sinks in either 307C or 306A and refilling with 100% bleach up to 20% of the flask.
16. Changing tubing and filter units (provided in the storage room 303A) for the vacuum flasks.
17. Bleaching dishes that are contaminated (or for any other reason) in 307C sink and disposing of them in a timely manner.
18. Peeling off a sheet of the floor sticky pads in front of or inside SRT305A, 305B, 306A, 307C doors daily or when dirty. This is to minimize the chance of contamination in the labs.
19. Disposing of all the cardboard boxes in the lab. We recommend not bringing shipping boxes into the labs as they are vehicles for contamination entry.
20. Refraining from giving unauthorized people key(s) to the labs or other rooms.
21. Decontaminating the hoods and incubators that they use annually or in the event of a major contamination.
22. Defrosting the freezers that they use. Please notify SCAC personnel, so we can watch out for Isensix alarms and possibly deactivate for the duration of the defrosting.
23. Transferring frozen vials in Mr. Frosty containers supplied by the Core at -80C to LN2 within 2 days and returning the Mr. Frosty containers to room 307C to ensure availability for other users. If usage will exceed four Mr. Frosty's, please consult SCAC staff.
24. Testing each PSC line for mycoplasma every 4 months for continuous culture. Notify SCAC staff if positive.
25. Notifying immediately SCAC personnel of any major problems such as contamination or equipment problems.

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SCAC RECOMMENDATIONS

PSC FEEDING, PASSING AND FREEZING

1. PSCs must be passed or used \leq 70% confluent.
2. PSCs must be passed at a ratio that will require 4 -6 days to reach 70% confluency. This will require splitting at various ratios. Err on the side of plating at low density so colonies can mature prior to reaching 70% confluency.
3. Time that PSC cultures are out of the incubator should be minimized. Procedures requiring more time should be done in smaller batches.
4. Use only actively growing, unstressed cultures (~day 4-5 culture, \leq 70% confluent) for freezer stocks.
5. Optional-A log of tissue culture activities should be kept daily. Cultured cell lines can be photographed to have as a record.

MEDIA HANDLING

1. Media supplements must be stored as manufacturer-recommends, and if frozen, thawed on ice or at 4C (exception for DMSO containing aliquots), and used by the expiration date.
2. Complete media should be made cold and thoroughly mixed (avoiding bubbles) before aliquoting.
3. Complete media stored at 4C/-20C should be thoroughly mixed before use.
4. Warm **only** the amount of media required for the current tissue culture session.
5. mTeSR media should be warmed to room temperature and HES media warmed to 37C before feeding cells. Other media to be treated as per manufacturer's recommendations.
6. Media should be protected from light upon warming and any extended time in the hood, which means overhead lights should be off when not using.

We is referred to SCAC (Stem Cell Analytics Core) personnel.

User is referred to any laboratory that is renting space in SCAC

Please contact **Manager-Narine Harutyunyan** and **Research Specialist-Andrew Salas** with any questions or concerns.

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Narine's cell phone: (323) 445-0240

Andrew's cell phone: (760) 646-2047