CDB Equipment Resource Guide

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Introduction

This manual contains both specific and general guidelines to using equipment provided by the Department of Cell & Developmental Biology at Vanderbilt University. Some of the instructions are critical and are presented with this menacing image:

This image means you may harm your experiment if you don't understand what is written. It is easy to use equipment without any comprehension of why or how it actually works, in the same way you can make hamburgers at McDonald's all day and not know how to cook.

General Rules Regarding All Equipment

It's a shared resource so leave the equipment ready for the next person.
The computers are also shared. Please see website for Data Retention & Management Policy.
For equipment with a fee attached, the PI has to supply users with a current, appropriate 10 digit center number. Users are responsible for correctly recording their usage.
Please tell the equipment manager if there is a problem, don't assume anyone else has reported an issue.

What is not in this Manual

You will not find step by step instructions for use of most equipment in this guide. Experience has shown that demonstrating how something works is more reliable than using these types of instructions. Over the long term, actually understanding the machinations of the instrument instead of following steps mindlessly will save you time, your lab money, and help you make wise decisions in your later career.

When things break

Please contact me immediately when something is wrong. I have my contact information on all equipment tags so that even during the evening you can get in touch with me. Ice that's black, melted autoclave trays, leaking water purifiers, lights out in the darkroom, anything… please call.

Abuse of Instrument

**NOTICE: If it is determined that a Resource User has abused an instrument, failed to follow established procedures or not cleaned up the instrument (i.e., such as cleaning under the stage down to the motor when flasks break in the incubator shakers), then the user's PI will be responsible for the repair.

The Equipment Resource Website

The information found here can also be found online at the CDB Equipment Resource's website with the addition of downloadable user manuals where possible.
https://lab.vanderbilt.edu/cdb-core-lab/

Autoclaves

Locations: MRB3 3159, 4159; MCN CC-2203

The autoclaves are heavily used in our department and it is important to move loads in and out efficiently. Feel free to remove the previous user's finished materials from the autoclave and put it on their cart or a counter. Please set a timer so you can show up near the end of the cycle to unload.

Below is what I consider an "aspirational" list of safety precautions. For example, rarely does one see an autoclave user wearing a rubber apron. However, these safety tips point to the very real dangers that high temperature liquids pose to users and great caution should always be exercised while unloading an autoclave.

Recommended practices:

Before using the autoclave, check inside the autoclave for any items left by the previous user that could pose a hazard (e.g. sharps).

Check and if necessary clear the drain strainer before loading the autoclave (little metal basket, front and center at bottom.).

Load the autoclave properly as per the manufacturer's recommendations (do not heap).

 To prevent bottles from shattering the caps of containers with liquids must be loosened before loading.

Use a POLYPROPYLENE (PP) tray with a solid bottom and walls to contain the contents and catch spills.

Add 1/4 to 1/2 inch of water to the tray so the bottles will heat evenly during liquid cycles.

Check plastic materials to ensure they are compatible with the autoclave (no LDPE marked items).

Individual glassware pieces should be within a heat resistant plastic tray on a shelf or rack and never placed directly on the autoclave floor.

Make sure the door of the autoclave is fully closed (latched) and the correct cycle has been selected before starting the cycle (run temperature should be 121°C).

Wear heat-resistant gloves when opening the autoclave door after a cycle. If there is a sharps hazard (e.g. biological waste), wear heat AND cut resistant gloves.

Before removing autoclaved items, try to wait 5 minutes for loads containing only dry glassware, and 10 minutes for autoclaved liquid loads.

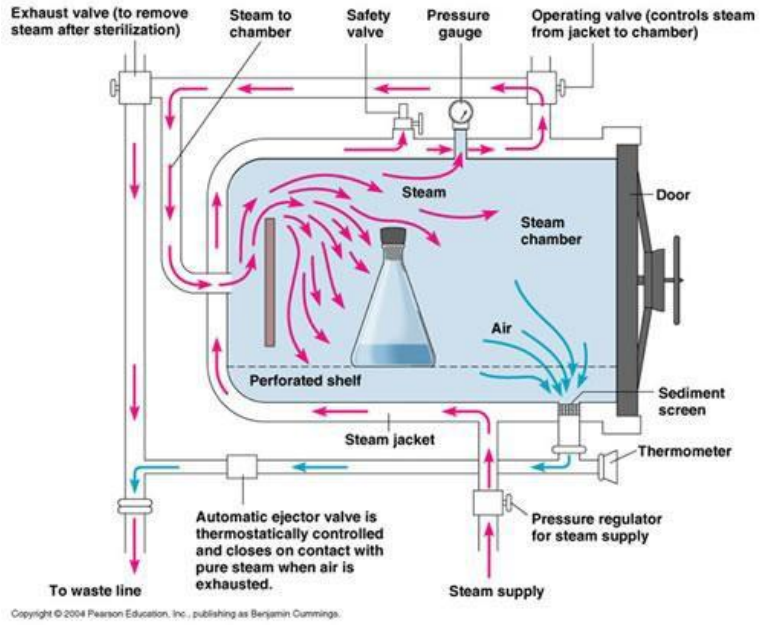
When removing items from an autoclave, a rubber apron, rubber sleeve protectors and heat-resistant gloves should be worn.

For non-liquid loads, let the glassware cool for 15 minutes before touching it with bare hands.

For liquid loads, let liquids stand for a full hour before touching with ungloved hands. Be sure others in the area know a heat hazard is present.

 Our autoclaves are downward displacement models. They work by forcing steam in at the side or back of the chamber which displaces the air downwards and out through a grated drain. There is turbulence inside the chamber (the steam comes in at about 25 p.s.i.) but solid walled trays can create a

tub of air which prevents steam from contacting the entire surface area of the bottles. This means they are not exchanging heat properly and the media may not reach sterilizing temperatures.



How a downward displacement autoclave works

Trays with holes would do a better job of reaching sterilizing temperature (Gillespie & Gibbons, 1975), but trays help with user safety and for keeping the machines in good working order. About a quarter of autoclave trouble calls are due to clogged drains according to the SER shop, sometimes because of agar media, sometimes because of melted trays, sometimes just built up detritus. Also, removing one tray with 16 flasks is much safer than directly handling these bottles of hot, scalding fluids individually. Finally, in the very unlikely event that the flasks explode, the walls of the tray provide you with some shielding. In the end, your safety is much more important than the sterility of your media so I encourage use of trays.

Centrifuges

Ultracentrifuges

Locations: TL-100 (tabletop), L90K MRB U-3202; L8-70M MCN B2318;

I want every student to get training from me before they use the ultras. Training takes about an hour. But the most important step in troubleshooting an ultracentrifuge run is figuring out the total volume and the volume per tube/bottle. The volume you need to spin will dictate which rotors you want to use. The rotor will dictate the tube and bottle type and with this knowledge you can get down to figuring out how long and what speed to spin. Don't get hung up on needing a particular rotor cited in the literature.

Super Speed centrifuges

Locations: RC-6 MRB3 3100 corridor; RC-5B MRB3 4100 corridor

The department has two Sorvall superspeed centrifuges, one which is modern, reliable, and under contract and one which has frequent breakdowns. Use the RC-6. Rotors for the RC-6 are kept in the 3143 cold room. We currently have SLC-4000, SLA-1500, and F21 8x50y rotors. Rotors for RC-5B are in 4143 coldroom. We have SLA-1500 and the older F21 model of rotor.

Control panel of RC-6

⚠ It is very important to tighten the lid to the rotor OUTSIDE OF THE

CENTRIFFUGE. When the lid is nice and snug, lower the rotor onto the drive shaft and tighten the rotor to it. Do not, ever, put the lidless rotor in the centrifuge and then attach the lid. This can cause a catastrophic rotor failure. In fact this has happened three times in the last four years. Again, I prefer a direct training on these machines as it only takes about 5 minutes.

If you encounter a problem with the RC-6, pressing the "CE" button usually helps ("clear error").

High Speed Centrifuge

Location: J-6B MRB3 3100 corridor

The J-6B is for large volume, relatively low speed centrifuge runs. The installed swinging bucket rotor only goes to 3,000 rpm, and the buckets have adapters for 50 and 15 ml conical tubes. Sometimes you need to hold the start button and hit the latch area at the same time.

Rotors (ultracentrifuge)

General use notes for rotors

1) Rinse rotors with deionized water after use and dry upside down.

Why: The biggest problem a metal rotor will encounter is not being cleaned after use. Ionic solutions (i.e. bacterial media and cesium salts) will corrode aluminum rotors.

Even the titanium ultraspeed rotors (like the Beckman 45 Ti) have aluminum lids and tops which can corrode.

Most of our ultracentrifuge rotors

2) Always balance rotor tubes on a balance or scale. Do not balance tubes by eye. Specifically do not balance bacterial growth media for cell pelleting by eye.

Why: Your eye is highly sophisticated, but it is not an analytic measurement device capable of discriminating fractions of a gram. Even small imbalances are harmful to the ultraspeed floor model centrifuges. In large tubes, very small differences in solution height can have very different liquid masses. Also, tubes and adapters have similar, but not identical masses. You may safely assume the buckets of any rotor are balanced if they are put in the correct swing slot.

3) Humidity is the enemy of centrifuges.

Why: Humidity causes condensation on the cool lining of a centrifuge. This additional thermal mass makes temperature adjustment less efficient. It also can get into the pump of ultracentrifuges hampering vacuum formation.

4) Rotor well adapters (such as for Falcon tubes) should be removed after every run. But since nobody does this, how about just once in a while?

Why: Because small amounts of sample will collect at the bottom of the well in between the adapters and the rotor. One technician told me that the worst corrosion he sees is caused by never checking under the adapters. Also bacterial growth media left in centrifuge rotors will (wait for it) grow bacteria in the media, which is rather disgusting for the next user.

5) If the rotor is heavy use a cart.

Why: So you don't drop the rotor, hurting it, yourself, your experiment, or someone else.

Cryotank

Location: MRB3 3100 corridor, outside of glasswashing room

Use of the cryostorage tank is subject to availability. The tank is set up to have a maximum volume of 6 inches from the bottom of the tank and a minimum volume of 3 inches from the bottom of the tank. The temperature reading on the console is measured at 23 inches above the bottom of the tank. Liquid nitrogen is −196 °C and tank temp usually measures around −130 °C, so tank temperature is a gradient of temperatures between these two points depending on the height of the stored sample. The bottom box of a tower will be submerged in liquid nitrogen at all times, while the second slot from the bottom will experience the rise and fall of the liquid level. All other tower slots are vapor stored.

The departmental cryotank

Because spills into the liquid nitrogen are nearly inevitable, I recommend preparing all samples for liquid immersion. Also, cardboard storage boxes of all types tend to become brittle and break with repeated freeze thaw cycles. Metal or plastic storage boxes are less likely to break and spill samples into the bottom of the tank.

Large towers hold up to 1,300 samples (13 slots, 10x10 boxes) and small towers hold up to 325 samples (13 slots, 5x5 boxes).

Dehydration Systems

Locations: SpeedVac MRB3 3159, MCN B2318; Labconco Freeze Dryer MCN B2318


SpeedVac

CDB has two types of dehydration systems - two Thermo SpeedVacs and a Labconco Freeze Dryer (Lyophilizer). The SpeedVac spins epi tubes at low pressure either heated or at room temperature. The freeze dryer works at very low temperature (-50 °C) and pressure to sublime frozen solvent. If you are wondering about which system to use, please contact the equipment manager. Both of these devices have their entire instruction manual for download on the equipment wiki.

Dry Ice

Location: MRB3, U-3202

Dry ice is kept in the equipment room in a gray chest cooler next to the entrance. Shipments arrive Monday and Thursday. We have two extra gray chest freezers which are useful for transporting samples during freezer de-frostings. Please tell the equipment manager if you use a rolling cooler.

Freezers

 **Under no circumstances should the departmental freezers be moved!** Just like any other equipment, moving them around increases the chances of damage to electronics, compressors, and copper tubing. Ultra-low -80 freezers are expensive and take a long time to get repaired. They need to be treated with the best of care to ensure that they are available for the next lab's emergency.

If you have an emergency thaw, call me immediately. Please go to the equipment room, grab the rolling grey freezer chests and use them to transport your endangered samples to one of the departmental freezers. Feel free to put some dry ice in the bottoms of the carts if you have to transport your goods a long distance.

Locations: 3100D and 4100B corridor MRB3 (-80s), 3149 MRB3 (-20), 4100D MRB3 (-20), 3201 MCN (-20 & -40)

The freezers (up to date locations on main equipment webpage) are for short term, emergency or project storage. Typically they are used for thawing freezers, intentionally or otherwise.

Glasswashers

Locations: MRB3 3159, 4159; MCN CC-2302

Preparing the Glassware

1. Dispose of solvents, buffers, media in the correct manner.
2.  Gel residue (agarose or polyacrylamide) should be very minimal or absent on dishes to be washed. Due to the recirculating nature of the machines, gel residue can be spread on all of your glassware by the normal washing cycle.
3. Tape should be removed, tape bits and residue can be spread by the normal washing cycle. The drying cycle can bake these bits of adhesive effluvia to a concrete consistency.
4. Encrusted and dry chemical residue may not come off under a normal cycle. It is best to soak dried or cooked residue before a washing.

Loading the Washer
There are several types of rack to choose from. Racks with fewer tines can clean larger pieces of glassware. This table has the recommended capacity for the tine racks (called spindle headers by Steris):

Rack	Erlenmeyer Flask	Graduated Cylinder	Carboys & Bottle Volume

Size	Volume	Volume	
2 x 4	500 – 1500 ml	250 – 500 ml	500 – 4000 ml
3 x 6	250 – 300 ml	25 – 250 ml	Not applicable
4 x 8	50-250 ml	25 – 100 ml	Not applicable

These are much smaller volumes than what is typically put into the glassware washer, but larger volumes seem to work. For beakers, try to sit them over multiple tines if they are large, or put them in the basket attachment if they are small. The basket (top left) should have the mesh metal cover placed on it during the wash cycle to prevent lightweight plastic items from being tossed into the bottom of the washer.

⚠ I do not recommend machine washing 4 liter graduated cylinders; the water does not reach the entire length of the cylinder.

⚠ The water is delivered from the hole at the top of each rack tine, so you can only put one piece of glassware on each tine.

When loading the washer, work back to front. The water is delivered by a series of gaskets. Each tine rack has a gasket that must fit over a corresponding supply gasket from the washer to operate correctly. See the pictures for various possible problems.

Dry

Each wash room has a glass dryer located opposite the washers which can be used for glass or autoclave load drying.

Glass Washing Service – None currently

Ice Machines

Locations: MRB3 U3200BA (near Macara cold room), 3159, 4100 corridor; MCN B2318

Each departmental ice machine puts out about 500 lbs of ice per day with holding bins of either 322 or 420 lbs. Since the newer machines have been installed we have not ever run out of ice. If you notice an empty ice machine, please contact the equipment manager because it is very likely broken. Also note that although these are restaurant models the ice is not safe to eat as we have no regular sanitation regimen.

Incubating Shakers

Locations: MCN – Innova 4430R and 43R (chest), B2318
MRB3- Innova 4430R, 3100A, 3100F & 4100C corridor

Shaker Guidelines:

1. Make sure the shaker begins shaking before you walk away.
2. Do not change the temperature or speed if there are any other samples in the shaker.
3. All Innova shakers except for 3100A must be reserved online. The 3100A is kept at 37 degrees for starter cultures.
4. Use the clamps provided to properly fit flasks. Do not use paper towels or detritus to secure flasks.

Innova 4430 shaker

5. Really, make sure the shaker is moving before you leave.
6. Arranging large volume flasks on the shaking platform so that they are symmetrical about the center. This practice reduces wear and tear on the shaker bearings.

⚠ Using Styrofoam or paper towels in the shaker is a significant fire risk according to the repair technician. There are MANY extra clamps and racks in the equipment room if you do not see the proper size.

Downloadable manuals with complete details on how to program the device are available on the wiki. All of our New Brunswick Innova shakers can refrigerate. One quote from the technician on temperature issues, "Do you really think that eight dollar alcohol thermometer is a better gauge than the thermocouple in your fourteen thousand dollar shaker?"

Isotope (scintillation) Counters

Locations: Gamma 5500 Counter 3141 MRB3, LS6500 Scintillation Counter MRB3 3149;

The very basics of liquid scintillation counting

The "LS" in two of the above models stands for "liquid scintillation". Samples emit β radiation, the energy of which is absorbed by the liquid medium (typically toluene or some other aromatic with many π bonds) which then bounces around until it hits a phosphor in the cocktail which finally releases the energy as light. The counter has two photomultiplier tubes (PMTs) on either side of the sample tube. If both register a light event at the same coincidence, it is not considered background and is registered as a count.

The LS-6500 in MRB3 3149

⚠ Because of the PMTs you should close the lid to eliminate outside light.

On the LS counters there are many preset programs, one of which almost certainly will fulfill your needs. I recommend using the automatic counting mode (described below) – just select the card corresponding to the program (cards are kept in a tray at the front bottom of the counter). Contact me if you need help editing a new count program.

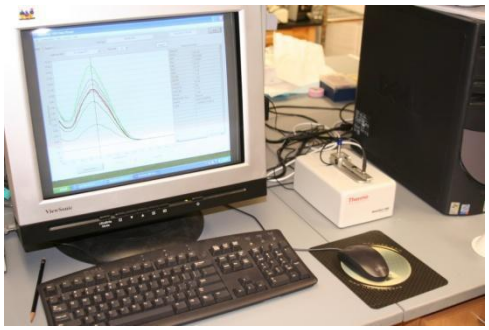
Using the automatic count function

1. Select the desired rack size, depending on the size of sample vials used, and install the command card/User No. card into the left set of slots on the first rack. This type of card has a long tab sticking up.
2. Load the rack with samples as follows:
    a. If blanks or replicates are not used, the samples may be loaded in any manner desired.
    b. If blanks are used, they must be loaded first followed by an empty space.
    c. If replicates are used, the replicates must be loaded in adjacent positions. If one or more replicates are missing from a group, leave only one empty position; the system recognizes the vial following the empty space as being the first replicate of the next set.
3. Place the loaded racks into the scintillation counter, beginning on the right side, as follows:
    a. The first rack must have the User Number Card installed that corresponds to the program you wish to run. This rack should also include any blanks.
    b. All of the following racks will be counted using the same program, unless a new User Card is encountered.
    c. Place the red Halt Rack behind the last sample rack.
4. Check to make sure the printer is on (the ON light and the ON-LINE lights should be lit) and that paper is loaded.
5. Using the arrow keys, highlight Automatic Counting, and press Select to begin counting.
6. If all of the samples have been loaded according to the instructions on the screen, press Start.

7. The results of the counts will be printed as they are completed. The sample in progress can be stopped by pressing the Stop Count key (the scintillation counter will then proceed to the next sample), or the run can be stopped by pressing the two Reset keys simultaneously.
8. To briefly stop the program to count a small separate batch of samples (up to one rack), press Select or Interrupt.
    a. The priority samples must be loaded in the Interrupt Rack.
    b. The data must be accessed after the run has been completed by selecting "Access interrupt Data" from the Main Menu, followed by "View Interrupt Data", and "Print Interrupt Data".

NanoDrop 1000 and 2000C

Location: MRB3, U-3202



NanoDrop - most popular piece of equipment in the core

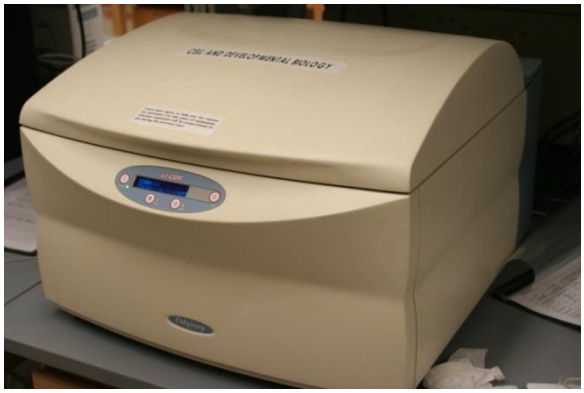
The NanoDrop is by far the most used device in the equipment resource. Although it can measure volumes as low as 1 µl for aqueous nucleic acid samples, I recommend 2 µl unless your sample is very precious. Evaporation becomes a real issue with only 1 µl, both by artificially concentrating your sample and for sometimes failing to form an adequate column of liquid for the beampath.

Because the NanoDrop works by drawing a column of water using surface tension, any type of detergent will likely prevent it from functioning properly.

Odyssey Laser Scanner

Location: MRB3, U-3202

The Odyssey is primarily used for scanning Western blot membranes with fluorescent secondary antibody tags. The Odyssey has two lasers which emit at 680 and 780 nm respectively so all secondary fluorophores should excite at one of these two wavelengths. The laser can also penetrate gels or microplates.



The Odyssey is waiting to scan your membranes

The greatest difficulty users have with Odyssey is the file system, because Odyssey only opens projects. The actual scan is a subfolder within the project folder and inability to discriminate between directory levels is by far the issue I troubleshoot most frequently. We have a copy of the Odyssey software I can install on almost any Windows computer if you wish to work in your lab.

The departmental wiki has many documents on successfully using Odyssey available for download.

Odyssey Hints & Tips

The following are some hints and tips to consider when making using IR Fluorescence for the first time. They were given to me by a Li-Cor representative:

It is extremely helpful to run a comparison experiment, with one blot in your traditional format (i.e. ECL) and the other with the IRDye protocol. This will allow for side-by-side comparisons to your current method.
Two-color detection requires primary antibodies from different host species.
Do not write on your gels with pen. Please use pencil.
If using blue ladder (MW Marker), use very small amounts (2-3 µL is sufficient to see on the Odyssey).
Do not add Tween to blocking buffer until after blocking.
Blocking can be performed using LI-COR Blocking Buffer (BB; recommended) or milk. Milk works well with nitrocellulose, but not so well with PVDF. Our BB may result in better sensitivity. If you have time, please try a blot in each, milk and Odyssey BB.
Do not use BSA for blocking; it decreases sensitivity quite a lot.
Adding Tween to antibody dilutions is recommended. Typically between 0.05% and 0.2% is adequate. 0.1% is most common.
Adding SDS to secondary antibody dilutions is recommended to reduce background and non-specific binding. For both PVDF and nitrocellulose membranes, between 0.01 – 0.02% is adequate.
Dilute antibodies in LI-COR BB (if used to block) or PBS/TBS.
Dilute and incubate primary antibodies as you typically do.
Dilute secondaries between 1:10,000 to 1:25,000. **To start, I typically recommend 1:20,000**.
Based on the results, optimum dilution can be tweaked. Incubation is for 1 hour at room temp.
Handle membranes carefully and with forceps. This is very important when using the secondaries. A rinse of the forceps in ethanol after use in secondary dilutions is recommended. I recommend a dilution volume of up to 10 mL for the secondary.
The dyes are stable. Therefore, membranes can be prepared and then stored either dry or wet at 4° C for scanning at a later time.
Drying membranes after secondary incubation, particularly nitrocellulose will yield higher sensitivity, but you will not be able to strip the membranes.
Be aware that the labeled antibodies are light sensitive. Therefore keep them covered when incubating (by covering the dish with foil or a box).
There are no special requirements or procedures for coomassie stained gels.

Plate Reader

Location: Synergy HT MRB3, U-3202

This device should have in person training, although it is not particularly difficult to use. The Synergy is multimodal and it can be used for most applications – it can read absorbance from 200-999 nm, fluorescence based on filters we have, and luminescence.

qPCR (or Real-Time PCR)

Location: MRB3, U-3202

The CFX96 Real-Time System from Bio-Rad is a quantitative PCR machine that must be reserved online. Software training on the CFX usually takes about half an hour. Make sure to purchase (or use/borrow) "low-profile" plates for this machine. We have a site-license for the software so I can install it on almost any Windows computer.

Typhoon 7100IP (phosphor plate reader)

Location: MRB3, U-3202

The Typhoon 7100IP

The Typhoon has one laser emitting at 532 nm for purposes of phosphor chemiluminescence. The instrument is upgradeable for other wavelengths if need is found. Most often the Typhoon is used for photostimulated luminescence, i.e. phosphor storage plate reading. Storage screens capture β radiation in the phosphor matrix and this energy is released when hit with a powerful light source such as a laser. A photomultiplier tube then captures the emitted light and records it on the scan.

Two major questions users have are:

1) Can this scan EGFP tagged (gel/membrane/other)? Answer – NO, but the LAS4000 can.
2) Can we use Fuji BAS-MS storage plates? Answer – YES

Ultrapure water

Locations: MRB3, 3159 & 4159; MCN B2319

I recommend using the on demand dispense switch at the dispenser handle as shown in the pictures.

Due to CO_2 absorption, ultrapure water is generally acidic – pH 5.8 or even lower. Also, without ions, ultrapure water cannot be accurately measured by a standard pH meter. Finally, diluting concentrated buffer

stocks with ultrapure water generally raises pH (a lot!) due to changes in ionic strength and buffer capacity.

X-ray Film Processors

Locations: MRB3, 3127 & 4125

⚠ Do not ever attempt to fix the film processors yourself.

⚠ Do not put cardboard film backers in the processor. Seems obvious but keeps happening. Cardboard gets dissolved in the developer/fixer solutions and chunks get in the recirculation line which ends up clogging the pumps. It costs $800 to have one of these pumps replaced.

Basics of the film processors

1) Drain water from the wash tub after use to prevent the growth of algae and mold in the dark, warm recesses of the processor. Remember to close the water tank valve before inserting films.
2) The developer and fix solutions come in **concentrated** form from the manufacturer. Each of those little plastic containers must be diluted to 5 gallons total. Please do not ever add solutions without dilution.
3) Films 8”x10” or smaller should be inserted lengthwise. All films should be aligned with the side of the tray as a guide. Putting film in the center of the tray can cause jams.
4) Because the fluids in the developers are heated, condensation often forms on the inside of the loading tray. Wipe away the condensation with a Kimwipe to avoid lines on your film.
5) The **minimum length** of film the processor can accept is 6 inches. Shorter films get caught in the rollers.
6) Films of 8”x10” or smaller may be inserted two at a time. Just line up one on each edge and make sure there is no overlap.
7) The loading tray should be **open** when not in use. Apparently with the modern solutions oxidation is not nearly the problem it was and leaving it open helps prevent condensation.

Note the wash tub knob in the “open” position. It should look like this when you leave. Make sure the knob is turned 90° before starting.

8) After 8 hours of inactivity, the processors shut off automatically. On weekends, late at night, or early in the morning you may have to turn the processor on (see Startup Procedure).

Startup Procedure:

If the machine’s lights are off, turn on the power (in the front, under the feed tray). When the power is on, the "Run" light will be lit.

Press the "Run" button. The tanks have to heat up before the "Ready" light comes on.

When both the "Ready" and "Run" lights are on, the processor is ready for use.

It will take up to 30 minutes for the solutions to warm during cold months, but it will stop heating after 20 minutes. Repeat the steps and it will get to temperature the second time.

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By Ian McCullough (v. 1.0)

Modified by Anthony Tharp (v. 3.0)