SOP for HTE plates
Written by Jessica Sampson, 16 February 2023

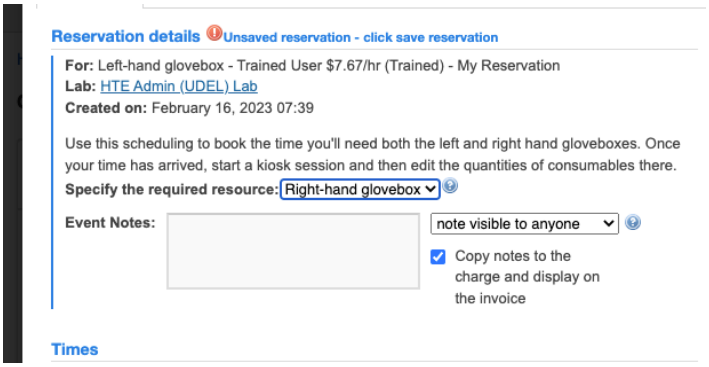
General and safety considerations:

1. It is the user's obligation to ensure that their reactions and workups are performed in as safe a manner as possible. If you have questions, please ask!
2. Highly toxic materials, strong acids and bases, flammable gases, pyrophoric reagents, etc. must not be handled while alone in the lab.
3. Solvents may not be used in the left hand glovebox.
4. Volatile protic solvents/reagents, volatile thiols and selenols, and volatile isocyanides must only be used in the glovebox with the catalyst off. Common reagent and solvent bottles must not be opened while they are in use or until after purging for at least 45 minutes. The catalyst must not be turned on until the box has been purged for at least 45 min. You must check with Jessica prior to using such reagents in the glovebox.
5. Liquid, volatile, and/or aqueous strong acids may not be used in the HTE gloveboxes.
6. Mercury, thallium, cadmium, tin, and lead reagents, volatile highly toxic materials, metal perchlorates, metal azides, and metal cyanides must not be used in the HTE gloveboxes without prior approval from Jessica.

Prior to running your plate:

1. Identify the variables that you want to screen against in your HTE plate. Variables could include ligand, solvent, catalyst, base, substrate, etc. but it is suggested to minimize the number of solvents screened in a single experiment since each one will require a separate set of stock solutions.
2. Identify the analysis method(s) that will allow you measure your desired outcomes, whether selectivity (ee or product ratios), yield, and/or recovered starting material.
3. Map out your variables onto the plate and plan out your experiment using the Excel template. Full guide to be written in the future.
4. Identify which chemicals you will need to supply for the study and weigh them out prior to the start of your glovebox time. A complete list of solvents and chemicals in the HTE gloveboxes is available at: https://docs.google.com/spreadsheets/d/1XRC7S7esnnfwu_7nb87P-vqFjOYLYYOb5ZV_WgR7Wuw
    a. If a chemical is not currently available in the HTE glovebox then speak with Jessica about how to bring it in and enter it into the inventory.
5. Book time in the HTE glovebox 16 h in advance of when you want to run the experiment. Time can be booked by going to: https://udel.ilabsolutions.com/sc/5897/high-throughput-experimentation-facility/?tab=equipment
    a. Select the "Schedule Resources" tab then "Reaction set-up"
    b. Next to "Glovebox" click "View Schedule" then drag and drop on the following screen to select the time you need. If you're going to be weighing out materials and collecting pre-plated ligands from the left-hand glovebox then you should make one

booking for that box then a second booking for the right-hand glovebox for the time required for liquid handling and purging. You can select which glovebox at the top of the reservation screen where it says “Specify the required resource:”



Day of running your plate

Materials required:

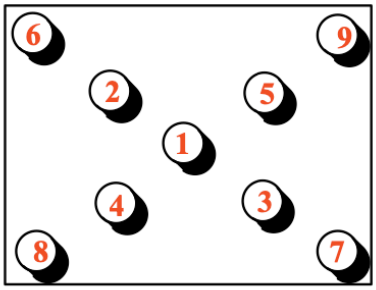
- Check the gloveboxes for the following and bring in if missing or low:
  - 8x30 mm glass shell reaction vials or pre-plated ligands in 8x30 mm glass shell reaction vials
  - Oven-dried PTFE covered stir bars
  - 250 μL and 1000 μL pipette tips
  - Pipettes:
    - 1000 μL single channel
    - 100 μL single channel
    - 50 μL 12-channel
  - Reagent reservoirs
  - Solid pipette and tips
- Collect the following from the benchtop to be brought into the glovebox:
  - 200 μL 12-channel micropipette (only required if the volume of one of the stock solutions is larger than 50 μL)
  - 24- or 96-well Paradox reaction block
  - Appropriately sized reaction block lid with two rubber mats and a PFA sealing sheet
  - Any chemicals not located in the HTE glovebox, weighed into the smallest possible glass scintillation vial
  - A vial tray for organizing stock solution vials
  - A plastic container for collecting your trash
  - Centering ring (if running a 24-well plate)
- PPE: lab coat, safety glasses, nitrile gloves

Procedure for preplated ligands/reagents:

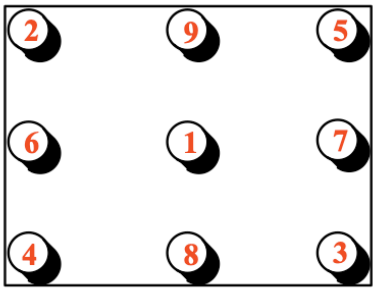
1. Enter all consumables you are using in iLab

2. Bring any benchtop materials into the left-hand glovebox, leaving under vacuum in the antechamber until cool to the touch (if hot) and cycling the antechamber with nitrogen at least three times (see glovebox SOP)
3. Weigh out any materials from the HTE glovebox into 4 mL glass scintillation vials and cap
4. Transfer preplated ligands into the Paradox block to the same position as you have recorded in your Excel planning sheet. If you are not running every reaction well then fill the blank positions with empty vials. Vials for this purpose are available around the lab if required.
5. If you are using an inorganic base and adding this by solid pipette, then this should be added to the appropriate wells at this point according to the solid pipettor method
6. Cycle the large antechamber (see glovebox SOP) and transfer all of your materials into the right-hand glovebox.
7. Using the small tweezers, transfer stirbars into each of your reaction wells.
8. Turn on the hotplate you are going to be using to the required temperature for your reaction.
9. Turn off the glovebox purifier and oxygen analyzer.
10. Using a calibrated micropipette, add the required amount of solvent to each of your pre-weighed reagents, cap, and shake to dissolve. If your material does not dissolve, then add a stirbar and place on the small IKA stirplate to stir while you proceed with the rest of this procedure. Throw away the used pipette tips.
11. Retrieve a reagent well of the appropriate size and place it behind the Paradox block, ensuring that column 1 of the reactor is to the left hand and that row D or H is closest to you. Pour or pipette your catalyst solution into the reservoir and then throw away this pipette tip (if used). Add an appropriate number of pipette tips to the 50 μL 12-channel micropipette, then use this to transfer your catalyst stock solution(s) into the appropriate wells. Transfer your remaining stock solution, reagent reservoir, and used pipette tips into your trash container.
12. Cap the reactor with the metal lid, two rubber mats, and a PFA sheet and loosely tighten the corner screws, then place the reactor on a stirring element and mix at 500 RPM for 20 minutes at ambient temperature.
13. Remove reactor from the mixer, then remove the lid from the reactor.
14. Using an analogous procedure as in step 9, pipette in each of your remaining required reagents, making note of your order of addition in the Excel sheet or your notes.
    a. Suggested order of addition: catalyst precursor, substrates, base, then reductant
    b. If you have two reagents that will react deleteriously, then the most reactive of those (likely the reductant) should be added <u>last</u> and as rapidly as possible
    c. If any reaction components are insoluble then they should be pipetted in well-by-well as a slurry (method description later in this SOP)

15. Seal the reactor with the metal lid, two rubber mats, and a PFA sheet, then insert screws and hand tighten according to the following pattern:



Screw pattern for
24-Well Reaction Plates



Screw pattern for
96-Well Reaction Plates

    a. If you think this is requiring too much force then the screw is like misaligned. Remove the screw, reset its position, and try again.
    b. Once everything is in place, use the electric screwdriver with a torque setting of 3 to do two rounds of tightening in the same pattern
16. Place the reactor on a preheated stirrer at your required temperature and stir at 500 RPM for the required reaction time. Use a piece of white electrical tape and a Sharpie to label your reactor with your initials and notebook number, the desired temperature, and the desired stir rate.
17. Remove all of your trash from the glovebox and dispose of it appropriately.
18. Purge the glovebox for 20 minutes, then turn on the catalyst and analyzer.
19. After your reaction time is over, remove the reactor from the hot plate, turn off the hot plate, and allow the reactor to come to room temperature, then remove from the glovebox.
20. Prepare a solution of your internal standard in the required solvent in the meantime.
21. If required, remove the reactor lid and add a stock solution of acid, reductant, etc. with a micropipette to quench your reaction, then stir the plate for 20 min, capped.
22. Remove the reactor lid and add your internal standard stock solution to each well with a micropipette, then carefully re-cap the reactor and allow to mix with stirring for 10 min.
23. Remove the reactor lid again and transfer aliquots into an analysis plate, then dilute with the required amount of fresh solvent and cap with a PTFE sealing film.
    a. For UV detection on LC-MS or SFC-MS, or GC-MS analysis, aliquots should be taken such that the maximum concentration in the heaviest, highest concentration reaction component is less than 0.5 mg/mL.
    b. For GC-FID analysis, aliquots should be taken such that the maximum concentration of your desired analyte(s) is around 1.5 mg/mL.
24. If high dilution is required for MS analysis, take a second set of aliquots from your first analysis plate, transfer them to a second, and dilute those with fresh solvent.
25. Centrifuge your final analysis plate for 15 min, following centrifuge SOP.
26. Submit your plate for analysis according to that instrument's SOP.
27. The same day that you take aliquots, transfer all of your reaction vials into a plastic vial holder and take them to your fume hood for storage, removing the stirbars at the same

time. Return the stirbars to Jessica unwashed or (preferably) rinse them twice with water and then stir them with aqua regia overnight. Rinse afterwards with water (2x) and acetone or isopropanol (2x), then transferring into a 20 mL glass vial and putting in the HTE oven. Label the vial with the time when you placed them in the oven.

Procedure for non-preplated ligands:

1. Start your kiosk session and enter all consumables you are using.
2. Bring any benchtop materials into the left-hand glovebox, cycling the antechamber with nitrogen at least twice (see glovebox SOP)
3. Collect a tray of hot vials from the oven and cycle into the glovebox, leaving in the antechamber under vacuum until cool to the touch.
4. Weigh out any materials from the HTE gloveboxes into 4 mL glass scintillation vials and cap
5. Invert HTE vials from the original tray into an empty plastic vial tray (there should be at least one in the glovebox), then invert again into the Paradox block.
6. Remove the tray used to heat the vials from the glovebox and fill it with fresh HTE vials while wearing gloves, then transfer it to the oven.
7. Proceed to follow steps 5 and forward as in the procedure for using preplated ligands.

Solid Pipettor Method:

1. Transfer a 4 mL vial to the balance and tare.
2. Add a pipette tip to the pipettor. Use the smaller (R1) for masses less than 5 mg and the larger (R2) for masses in the order of 6-15 mg; note that these are only guidelines and the other may be required depending on the solid properties.
3. Set the pipettor to a measurement of 2, then firmly but not forcefully press it into your target solid twice, then dispense this into the tared vial.
4. If the reading is larger than the target mass, then decrease the setting on the pipettor; if the reading is smaller than the target mass, then increase the setting on the pipettor.
5. If solids are sticking to the outside of the pipette then use the static gun on the tip, solids, and vial
6. Iterate on this process until you are able to dispense the target mass, taring the balance in between each attempt.
7. Once you have achieved one good reading, use the same settings twice more and record the masses you dispense in your notebook and the pipettor settings you used, including the reading on the dial, the tip size, and the number of presses. If the variability of your dispense is within your comfort level, then you can go ahead and dispense into your target HTE vials using those settings.
8. With some solids you will likely find that increasing the settings on one of the tips does not cause more material to be picked up or leads to large variability in the dispense, in this case you may need to swap the sizes on the pipette tip in order to dispense reliably.

Sometimes this will mean that multiple dispenses of the same solid need to be performed such that the exact target mass can be achieved.

9. If the solid pipettor tip requires cleaning, then remove it from the glovebox, remove the plunger from the barrel and rinse both components with DI water for several minutes, then with isopropanol. Allow the pieces to dry separately at room temperature overnight, then bring back into the left-hand glovebox

Slurry Pipetting Method:

Note: Stock slurries should be placed in a 4 or 20 mL vial with a sufficiently large stirbar such that you can achieve a good vortex. The size of the opening on the 8 mL vials makes it challenging to pipette out of them but they can also be used if necessary, it just takes a bit more effort.

1. Weigh out your material into an appropriately sized vial, preparing around 1.5 times as much as you need for your target vials, then add a stirbar.
2. Add your reaction solvent in the right hand glovebox with the catalyst off using a calibrated micropipette, then cap the vial and place it on the small IKA stirplate with a stir rate of 1000 RPM.
3. If the solids are finely divided and you can see them well dispersed throughout the liquid level then you can proceed to the next step, however, if the top of the liquid level does not contain the solids then transfer everything to a larger vial.
4. Add a pipette tip from the box labeled “Slurry pipette tips” to the micropipette, then, while stirring the parent mixture at 1000 RPM and with the vial centered on the stir plate, pull the mixture into the tip. Do this twice more to check whether or not the tip is successfully filling or whether you need to select a tip with a wider aperture.
    a. Note: the ends of the pipette tips in that box have been cut off with scissors by hand, so there is a lot of variability in the width of the opening. Solids with larger particle sizes may clog some pipette tips with smaller openings.
5. Pipette out of the parent mixture into each well that requires that material. To help keep track of your position, verbally count out when you pull up material into the pipette.