

Harvester Protocol

Using the Chen Lab Harvester

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Purpose of using Harvester:

- Can harvest reactions out of the 96 well block (large white blocks that hold 2ml each)
- Filtration with liposomes
- Uptake assay=proteoliposome + radioactive substance
- Suck up reactions and put them on a filter paper
- Can wash exogenous stuff with the wash buffer

Steps:

- 1.) Fill the large bottle on the left side with Wash Buffer
- 2.) Place the special filter paper inside. Makes holes in paper on the top and side since it is larger than the area of holes it has to cover. Make sure each hole is completely covered.
- 3.) Wet the filter paper with water before closing the lid.
- 4.) Close the lid on top and clamp it shut. Each hole needs to seal with an O-ring so that each sample stays separate.
- 5.) Turn on vacuum with the switch at the back
- 6.) Turn on the pump with switch at left side
- 7.) Hook up waste container on the right side with the correct colored stickers (purple and black). Make sure the top is sealed well, screw cap on. This waste container collects radioactive waste inside liposomes.
- 8.) Rinse everything with water using the wash plate. There are 2 needles: one deposits buffer in the well and the other sucks it up
- 9.) There is another switch on the side with 3 options:
 - a.) OFF: dispenses liquid thru needles but won't suck up (do this to wash)
 - b.) PURGE: do this first; washes out whole system with buffer and won't go to the plate
 - c.) HARVEST: dispenses AND sucks up. To dispense, push on the button that is on the thing with many needles. To suck up, release that same button.
- 10.) Purge the system with water first.
- 11.) Switch to off to dispense from needles

12.)Harvest

13.)Wash 3 to 4 times when dispensing on filter paper

14.)Remove each individual circle of filter paper and put it in scintillation fluid

Storage and Disposal:

-Can use Sodium azide to store all the little tubes to prevent things from growing inside the tubes

-When done: put water back in left bottle and purge and wash system thoroughly. Put down regular paper towels on the wells where filter paper normally goes in order to not waste the filter paper.