NATIONAL CRYSTALLIZATION CENTER

HAUPTMAN-WOODWARD MEDICAL RESEARCH INSTITUTE

Updated August 2023

Reproducing HWI National HTX Center screening hits using microbatch under oil.

Note that the order of addition (cocktail, sample, and oil) can be modified based on your sample viscosity and the time it takes to set a drop. If pipetting by hand, try different ordering of pipetting steps to see what works best in your hands. Sometimes pipetting the oil first and then dispensing onto the bottom of the well works best, sometimes adding protein to the cocktail and then adding oil one well at a time works best.

1) Create grid of optimization conditions around initial hits (vary pH, concentration, PEGs, etc).

2) Introduce small variations in cocktail/protein/precipitant/buffer/water ratio
   - Gradient screen pH 0.2-0.5 intervals
   - Precipitant concentration 2-5% increments (or 10-25 mM if a salt)

3) We typically use 1:1 ratio of cocktail: protein sample, but multiple wells on the plate can be used to vary the ratio, as well.
   - To vary drop volume ratio:
     Set up 3 wells using the following drop volume ratio:
     - 1microL protein + 2microL cocktail
     - 1microL protein + 1microL cocktail
     - 2microL protein + 1microL cocktail

4) We use Paraffin oil (PX0045-3 EMD chemicals)

There are two plate options for microbatch optimization:
MRC 96 (8x12) and Greiner 72 (6x12)

Using the MRC 96 SBS Format Plate

Available from Hampton Research (HR3-102):
https://hamptonresearch.com/product-MRC-Under-Oil-96-Well-564.html

1) Dispense 18 – 20 microL oil into each well.
   - If pipetting the cocktail and sample, recommend multichannel and using 18 microL oil. It can also be helpful to use a positive displacement pipette for oil delivery.
   - If using a robot for the cocktail and sample, can use smaller sample volumes so can use 20 microL oil.

2) Dispense 200 nL – 1 microL optimization cocktail using a robot or pipette.
   - If pipetting by hand, we typically use 1 microL cocktail drops.
   - If using a robot, smaller volumes of cocktail can be dispensed.

3) Dispense 200 nL – 1 microL optimization cocktail using a robot or pipette.
    - If pipetting by hand, we typically use 1 microL drops. When pipetting, dispense by touching the pipette tip to the cocktail drop (do not mix).
    - If using a robot, smaller volumes of cocktail can be dispensed using the syringes/pipette tips.

4) Cover the plate with seal (recommend UV-transparent seals for imaging with UV/SHG).

5) Centrifuge plate gently to merge the drops (~1000 rpm for a few minutes).

6) Note – if using multiple drop volume ratio numbers, have 32 cocktail conditions to work with on the 96 well plate.

Using the Greiner 72 Plate:

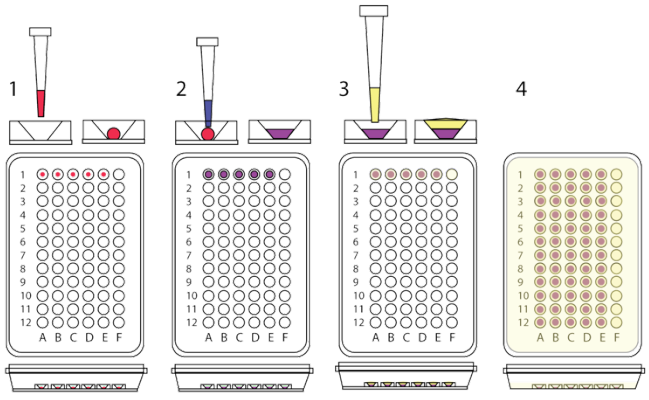
Available from Hampton Research. We typically use the treated hydrophilic (HR3-121): https://hamptonresearch.com/product-Microbatch-72-Well-Plate-Greiner-94.html

1) Add 1 microL cocktail drop to each well. You can use a microscope to watch the drop deliveries to ease set up if desired.

2) Add 1 microL protein drop (do not mix) by touching the pipette tip to the cocktail drop and dispensing the cocktail.

3) Add 20 microL of Paraffin oil to each well.

4) Once all the drops have been set up for a given plate, cover the entire plate with an additional 5-6 milliliters of the Paraffin oil.



5) Depending on speed of dispensing, can do one well by one well, or do multiple wells at a time. It can be helpful to use a positive displacement pipette for oil delivery.

6) Note – we typically do not use robots with the Greiner 72 Plate as it is not SBS format.

7) Note – if using multiple drop volume ratio numbers, have 24 cocktail conditions to work with on the 72 well plate.