

General EvoSep Tip Prep SOP

Monday, March 22, 2021 1:16 PM

Location of tips: EvoSep tip boxes are currently stored in the EvoSep cart. Dead tips are also in the cart, in a box labeled "DEAD TIPS (for balance)".

Materials needed:

EvoSep Tips
2 Empty EvoSep tip boxes
Dead tips for balance (if needed)
1 or 2 propanol (isopropyl alcohol)
EvoSep buffer A (0.1% formic acid in water)
EvoSep buffer B (0.1% formic acid in acetonitrile)
96-well plate with EvoSep white rack
Plastic reusable boats for buffers (labeled A, B, and ISO)

NOTE: since the MacCoss lab does not currently have a centrifuge suitable for prepping these tips, we are using the Beckman centrifuge owned by the Swanson lab down the hall. Make sure you have access to the centrifuge Google calendar and properly sign up for time. Carrying your supplies to the centrifuge in a bin is a good idea.

NOTE: it is wise to label things as much as you can. If the EvoSep boxes you are using to spin are not labeled, label them 1 and 2, and then on each box, front and back (or sample and balance, whatever works for you). Otherwise you will lose track of which tips are which in the centrifuge and you will be sad :-)

Prepping your supplies

Add buffers to appropriate boats

Using a multichannel pipette, add 100 ul of propanol to the appropriate number of wells in the 96 well plate (aka, the # of tips you are prepping)

Either divide the number of tips you are prepping evenly between the 2 boxes, or add the appropriate number of dead tips to the box not containing your sample tips

Bring everything to the centrifuge down the hall (leave the propanol boat behind, you don't need it anymore)

Setting up the centrifuge:

Make sure you have at least 2 swinging buckets for plates on the rotor

Make sure the appropriate rotor is selected in the program

Settings:

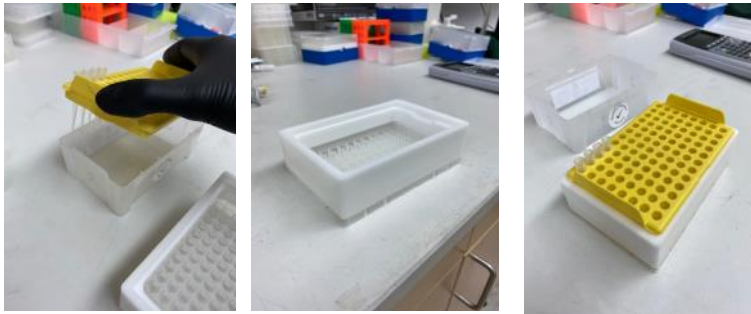
- Speed: 700 g
- Time: 00:01 (1 minute)
- Temperature: 20 C

Prepping the tips

Using a multichannel pipette, add 20 ul of buffer B to the tips

- Spin for 1 minute at 700 g
- Discard liquid in box

Soak the tips in the propanol for 30-60 seconds by gently pulling off the yellow rack-insert from the tip box, and setting it onto white rack on the 96-well plate, making sure the tips are submerged in the propanol



Make sure the tips have turned a semi-translucent gray from their original bright white



Replace the yellow rack-inserts back into their boxes

Add 20 ul of buffer A to the tips

- Spin for 1 minute at 700 g
- Discard liquid in box

Add your sample to the tips (this is where you really need to make sure you know which box is which and where your tips are!)

- Spin for 1 minute at 700 g
- Discard liquid in box

Add 20 ul of buffer A to the tips

- Spin for 1 minute at 700 g
- Discard liquid in box

Add 100 ul of buffer A to tips

- Hold down "pulse" button until centrifuge speed reaches 100-120 g, and then stop
- This spins the buffer down to rest on the tip material, to keep them from drying out, but does not spin it through

Clean everything up, disinfect the centrifuge, and your tips are ready to go!