

Concentrating Pathogens from Raw and Primary Wastewater Using the InnovaPrep® Concentrating Pipette™

Protocol (Revision D)



Introduction:

The InnovaPrep Concentrating Pipette Select (CP Select™) is an automated, bio-concentrator for rapid enrichment. The CP Select uses dead-end filtration with single use Concentrating Pipette tips (CPTs) to capture microorganisms from the fluid sample matrix. After filtration, the system uses a Wet Foam Elution process to recover the micro-organisms into a small sample volume in seconds. The system's ease of use and ability to deliver exceptionally high concentration factors make it an ideal approach for concentration of SARS-CoV-2 in wastewater (WW) as well as any other pathogen in a high turbidity matrix. Please reference the latest revision of this document on our website for the most current methodology.

SAFETY:

Due to the potential presence of infectious pathogens in wastewater samples, users should work with their organization's occupational safety team to ensure that methods and safety measures are appropriate and approved. Unless working with samples known to be non-infectious, InnovaPrep recommends that CP Select operations be performed in a biosafety cabinet.

One source of information published by the U.S. CDC: *Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)*.

Materials Required:

- **CP Select instrument**
- **FluidPrep Prefilter** choose from
 - **0.9 micron** item # CC08101 for viruses
 - **25 micron** item # CC08121 for bacteria or simultaneous concentration of viruses and bacteria.
- **Elution Fluid - Tris** item # HC08001
- **Concentrating Pipette Tips (CPTs)** choose from
 - **Ultrafilter CPTs** – item # CC08003-10 (irradiated) **or** bulk discounted item # CC08004-200 (non-irradiated)
 - **0.05 µm CPTs** – item # CC08020-10 (irradiated) **or** bulk discounted item # CC08011-200 (non-irradiated) for more turbid wastewater samples or for larger volumes.
Or if you wish to simultaneously concentrate both bacteria and viruses using the 0.05 µm CPTs **See special settings instructions on next page.*
 - **0.2 µm CPTs** – item # CC08022-10 (irradiated) **or** bulk discounted item # CC08053-200 (non-irradiated) For bacteria samples up to 1Liter. ***See special settings instructions on the next page.*
- **CP Storage fluid** – item # HC08558 for system decontamination
- **Tween 20** - for sample addition

Step 1 – Select Tip type and Prefilter pore size

A CPT should be selected based on preliminary evaluation of your wastewater matrix and your selected pretreatment method. Irradiated and non-irradiated tips perform the same.

- Ultrafilter CPTs (slightly higher efficiency) recommended for sample volumes up to 50 mL
- 0.05 µm CPTs (Slightly Faster Processing) recommended for sample volumes up to 100 mL
- 0.2 µm CPTs – For bacteria samples up to 1Liter.

Step 2 – Preparing the Influent wastewater

1. Prepare a 10% Tween 20 stock solution (Tween 20 has been shown to significantly increase SARS-CoV-2 recovery from wastewater)
2. Add the prepared Tween 20 to the starting Influent wastewater sample at 1:100 (e.g. 1 mL addition to 100 mL influent wastewater).
3. Shake or vortex briefly

Step 3 – Concentration

1. Following the instructions provided in Section 9.5 of the CP Select User Guide, set up a Custom Protocol using the Advanced Options as shown below:

Protocol Name	Valve open ms	Pulse	Foam Factor	Valve Closed ms	Flow Start sec.	Flow end sec.	Flow min start sec.	Ext Delay sec.	Pump %	Ext pump delay sec.
COVID WW	770	1	10	100	3.0	10	40	3	25%	1

This protocol is designed to deliver sufficient concentrated sample volume to perform two nucleic acid extractions with standard kits (one for an initial run and one as an archive sample). Sample concentrate volumes should be optimized to minimize the mismatch between this volume and the nucleic acid extraction protocol input volume. The concentrate volume can be increased by increasing the valve open time and/or pulse count (for open times above 800 ms it is suggested that 2 pulses be used). A pump setting of 25% has been demonstrated to enhance recovery and is suggested for this application.

**To simultaneously concentrate both bacteria and viruses using a 0.05 um CPT and 25 um prefilter: Using a lower pump power setting improves recovery performance, but results in longer run times. We suggest using a pump power setting between 25% and 50%. For highly turbid samples pump powers of closer to 25% should be used, while higher clarity samples may be processed with pump powers closer to 50%. Volumes processed should be limited such that run times are less than 20 minutes.*

***For concentrating bacteria only using a 0.2um CPT and a 25 um prefilter: Start with the default HOLLOW settings then create a custom setting and adjust the flow end up to 15.*

3. Insert a CPT into a FluidPrep Prefilter and then insert the assembly in the tip port of the CP Select.
4. Lower CPT assembly into the sample.
5. Press the “Start Run” on the user’s screen. When the entire sample has been processed the CP Select will stop.
6. Press down on the head of the instrument to force the tip to puncture the foil seal in the prefilter.
7. Place a clean final sample container under the CPT. The menu screen will prompt you to press “Elute.”
8. Press “Elute.” The sample will dispense from the CPT into the sample container. The sample is ready for subsequent sample preparation and analysis steps.

Step 4 – Sample extraction and analysis method of choice (digital PCR/qPCR, digital RT-PCR/RT-qPCR, NGS, etc.).