

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

Protocol

Introduction:



The InnovaPrep Concentrating Pipette Select (CP Select™) is an automated, bio-concentrator that rapidly enriches microorganisms mechanically. 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

Materials Required:

- **CP Select instrument**
- **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.
 - **0.2 µm CPTs** – item # CC08022-10 (irradiated) **or** bulk discounted item # CC08053-200 (non-irradiated) For bacterial targets up to 1Liter. **See special menu settings instructions on the next page.*
- Prefilters (0.22µm) or Centrifuge (7000-10,000 g)
- **CP Storage fluid** – item # HC08558 for system decontamination
- **Tween 20** - for sample addition

SAFETY:

Due to the potential presence of infectious pathogens in 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.

Procedure:

Step 1 – Preparing the wastewater sample

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) to yield a final concentration in sample of 0.1% Tween 20.
3. Shake or vortex briefly

An optional sonication step, which has been shown to improve viral recovery efficiency, can be performed prior to centrifugation. To dissociate viruses from solids in the sample, the wastewater sample should be sonicated for 1 minute using settings of nominally 50 kHz, 100 W or the "high" setting. This can be done using a cup-horn adapter or by placing the sample container in an ultrasonic bath. However, it is not generally recommended to sonicate the sample for longer than one minute or more than once when targeting viruses.

Step 2 – Prefilter sample

When targeting small organisms such as viruses, a wastewater sample should be prefiltered or centrifuged.

- A 0.22 µm prefilter device may be used or
- centrifuge for 10 minutes at 7,000 to 10,000 g. If a lower g-force is used (e.g., 3,500-4,000 g), the centrifugation time should be increased to 20 minutes or more. It is important to optimize the g-force and time for the specific sample matrix.

However, when targeting larger organisms such as bacteria, it is recommended to avoid centrifugation or prefiltering (unless using larger pore coffee-type filters) to prevent loss of the target organism. Instead, it is recommended to use 0.2 µm Concentrating Pipette tips (CPTs) for direct concentration from the wastewater without any sample pretreatment. This method can be used for sample volumes up to 1L, depending on the turbidity/suspended solids in the specific sample.

Step 3 – Concentration

Select a Concentrating Pipette Tip (CPT) from the following options:

- Ultrafilter CPTs (slightly higher efficiency) are recommended for sample volumes up to 50 mL
 - 0.05 µm CPTs (slightly faster processing) are recommended for sample volumes up to 100 mL
 - 0.2 µm CPTs – For bacterial and similar samples up to 1 L
1. Following the instructions provided in Section 9.5 of the CP Select User Manual, 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

The protocol is designed to provide a sufficient eluted volume of concentrated sample to conduct two nucleic acid extractions using standard kits (with approximately 200 µL input volume), allowing one for an initial run and one as an archive sample. For best concentration efficiency the total sample concentrate volume should be optimized to closely meet the total volume needed for extraction and archive sample(s). If necessary, the concentrate volume can be increased by increasing the valve open time and/or pulse count. If the valve open

time is above 800 ms, it is recommended to use two pulses. It has been demonstrated that a pump setting of 25% enhances recovery and is suggested for this application.

* When concentrating bacteria using a 0.2 µm Concentrating Pipette tip (CPT), it may be necessary to reduce the valve open time to reduce the elution volume, as well as increasing the flow min start-up time to prevent timing out. This will ensure optimal performance and prevent any issues at start-up.

2. Insert a CPT into the tip port of the CP Select.
3. Lower the CPT into the sample.
4. Press “Start Run” on the user’s screen. When the entire sample has been processed the CP Select will stop.
5. Place a clean final sample container under the CPT. The menu screen will prompt you to press “Elute.”
6. 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.).