version 25.04.22

# PathoSense

2025

CONFIDENTIAL



## Wet lab flow diagnostics PathoSense

# WET LAB I - Enrichment

### Viral enrichment via Benzonase-Nuclease

**Timing ~ 45 min**

PathoSense highly recommends to perform the enrichment step **daily**, so that samples are stabilised as soon as possible after arriving in the lab.

## Host ≠ Cat/Dog

### Reagents

- IC + HMB, provided by PathoSense, aliquoted by Partner Lab
- 10 nM EDTA, provided by PathoSense
- Benzonaze® Nuclease, Purity > 90%, 70746 Millipore = enzyme, so keep on -20°C!

| REAGENT | QUANTITY per sample | HANDLING CONDITIONS |
|---|---|---|
| IC+HMB | 1 aliquot | on ice |
| Benzonase nuclease | 3.5 µL | -20 °C (on cool rack) |
| 10 nM EDTA | 12.5 µL | on ice |

## Materials

- 1,5 mL Eppendorf tube
- 1,2 µm filter
- PathoSense Sample

- All surfaces should be cleaned with Hibiscrub, 70% Ethanol, and 10% Bleach in this order of cleaning. Prevent contamination.
- Aerosol-barrier tips should be used throughout the entire procedure.
- Perform all steps in the dedicated sample preparation area.

## Preparations

- Pre-heat the **heat block at 37°C**
- Thaw a 1.5 mL Eppendorf tube with **an aliquot FeCVII internal control (IC) + 20x Homemade Buffer (HMB)** on ice **just before adding the sample** (because the IC contains a virus)

## Protocol

1. **Label** a **1.5 mL Eppendorf tube** for each sample
2. Connect a **filter to the syringe** containing the sample
3. **Flush the sample through the filter** in the corresponding labeled Eppendorf tube
4. Take a **1.5 mL Eppendorf tube with an aliquot FeCVII internal control (IC) + 20x Homemade Buffer (HMB)** for each sample

5. Transfer **200 µL of the sample** to the Eppendorf with the aliquot FeCVII internal control (IC) and 20x homemade buffer
6. Add **3.5 µL Benzonase -Nuclease** to each tube
7. Gently **mix by inverting tube 3 times** (DO NOT VORTEX) and quick spin to collect all droplets
8. Incubate the tubes for **30 minutes** at **37°C**
9. Add **12.5 µL 10nM EDTA** to each tube to stop enzymatic reaction
10. Gently **mix by inverting tube 3 times** (DO NOT VORTEX) and quick spin to collect all droplets

**Safe stopping point**

If the samples cannot be processed immediately, Benzonase-treated samples can be stored at -70°C for 1 month

---

Keep Benzonase-treated samples on ice and proceed with

**WET LAB II EXTRACTION**

---

## Host = Cat/Dog

### Reagents

- HMB, provided by PathoSense, aliquoted by Partner Lab
- 10 nM EDTA, provided by PathoSense
- Benzonaze® Nuclease, Purity > 90%, 70746 Millipore = enzyme, so keep on -20°C!

| REAGENT | QUANTITY per sample | HANDLING CONDITIONS |
|---|---|---|
| HMB | 12.5 µL | on ice |
| Benzonase nuclease | 3.5 µL | -20 °C (on cool rack) |
| 10 nM EDTA | 12.5 µL | on ice |

## Materials

- 1,5 mL Eppendorf tube (2)
- 1,2 µm filter
- PathoSense Sample

- All surfaces should be cleaned with Hibiscrub, 70% Ethanol, and 10% Bleach in this order of cleaning. Prevent contamination.
- Aerosol-barrier tips should be used throughout the entire procedure.
- Perform all steps in the dedicated sample preparation area.

## Preparations

- Pre-heat the **heat block at 37°C**

## Protocol

1. **Label** a **1.5 mL Eppendorf tube** for each sample
2. Connect a **filter to the syringe** containing the sample
3. **Flush the  sample through the filter** in the corresponding labeled Eppendorf tube
4. Take a **1.5 mL Eppendorf tube** and transfer **234 µL of the sample**
5. Add **12.5µL 20x Homemade Buffer** (HMB) for each sample to the Eppendorf tube
6. Add **3.5 µL Benzonase -Nuclease** to each tube
7. Gently **mix by inverting tube 3 times** (DO NOT VORTEX) and quick spin to collect all droplets
8. Incubate the tubes for **30 minutes** at **37°C**
9. Add **12.5 µL 10nM EDTA** to each tube to stop enzymatic reaction
10. Gently **mix by inverting tube 3 times** (DO NOT VORTEX) and quick spin to collect all droplets

**Safe stopping point**

If the samples cannot be processed immediately, Benzonase-treated samples can be stored at -70°C for 1 month

---

Keep Benzonase-treated samples on ice and proceed with

# WET LAB II - EXTRACTION