Varizymes®
OPTIONS AMPLIFIED

AqPathDx™

Pathogen Test Kit - Panel 1 (Research Use Only)

Flavobacterium spp., *Aeromonas* spp., and *Edwardsiella* spp. DNA

Intended Use

The **AqPathDx Pathogen Test Kit - Panel 1** is a qualitative, real-time LAMP assay for the detection of *Flavobacterium* spp., *Aeromonas* spp., and *Edwardsiella* spp. DNA in fish samples, with an internal control (IC) to monitor sample and reaction integrity. It is intended for use by trained laboratory personnel in aquaculture or veterinary diagnostic laboratories.

Test Principle

This assay uses lyophilized LAMP reagents in 0.2 mL PCR tubes, combined with a simple lysis step and direct addition of clarified lysate to the reaction. Target amplification is monitored by real-time fluorescence in four channels corresponding to the three pathogens and an internal control.

Kit Components

Reagents are shipped on ice packs. For long term storage, **keep all reagents at 4 °C at all times**.

- Lyophilized **AqPathDx Test Panel 1 Foil Pouch** (x2)
- 10X lysis buffer
- **AqPathDx Kit 1 Reagent**

Additional materials required (Supplied by user)

- Real-time thermocycler with 4 or more fluorescent channels
- Water (DNase and RNase free, molecular grade)
- If testing samples: swabs and 15 ml tubes
- Mini vortex and centrifuge (optional)
- Gloves, appropriate pipettes, and tips
- Disposal bag

Storage and handling

Store all kit components at 4 °C; do not freeze unless otherwise validated. Keep reagents protected from light and do not use past the expiration date or if vials or pouches appear damaged.

Warnings & Precautions

- Always wear appropriate PPE and change gloves frequently, especially after handling samples.
- Perform sample preparation in a designated area, separate from reaction setup, to reduce contamination risk.
- Use only sterile, DNase/RNase free filter tips for all pipetting steps.
- Do not open reaction tubes after amplification to avoid amplicon contamination.
- Dispose of all biological materials and consumables in accordance with institutional and local regulations.

Preparation of 1X Lysis Buffer

1. Prepare 1X lysis buffer by adding 1 mL of 10X lysis buffer to 9 mL of molecular-grade water and mixing thoroughly. Scale volumes according to the number of samples to be processed.

Sample Collection & Preparation

1. Collect sample from diseased fish using a sterile swab. Transfer the swab to a 15 mL tube and add 2 mL of 1X lysis buffer.

2. Mix by tapping the bottom of the tube or vortexing for 5 seconds to release material from the swab.

3. Prepare dilution buffer:

a) **For 1 kit**: add 4 µL **AqPathDx Kit 1 Reagent** to 386 µL 1X lysis buffer

b) **For 2 kits**: add 8 µL **AqPathDx Kit 1 Reagent** to 771 µL 1X lysis buffer

4. Prepare a 40 fold dilution: add 1 µL of sample to 39 µL of dilution buffer from Step 3 and mix well by tapping or vortexing for 5 seconds. Scale volumes according to the number of replicates per sample to be tested.

Setting Up LAMP Reaction

Step 1: Each test foil pouch can test 8 samples. If stored at 4 °C, take out the test kit and keep it at room temperature for about 10 minutes.

Step 2: Open the foil pouch, remove the test strip, and place it on a 0.2 mL PCR tube rack. Gently tap the bottom of tube (or centrifuge) to make sure lyobead is at the bottom of tube. Label each reaction tube clearly to correspond with sample IDs, including positive and negative controls.

Step 3: Add 22 µL of sample (from Sample preparation Step 4) directly into each reaction tube containing the lyophilized bead.

Step 4: For the negative control, add 22 µL of 1X lysis buffer.

Step 5: Close all tube caps tightly to prevent contamination or evaporation. Mix gently by tapping at the bottom to tubes to remove any air bubble. A mini centrifuge may be used to spin down the lyo mix.

Step 6: Incubate the tubes at 68 °C for 35 minutes in a real-time thermocycler.

Step 7: Record results as per Table on next page.

Channel	Fluor	Detection
CH1	FAM	*Flavobacterium*
CH2	HEX	Internal Control (Fish Sample)
CH3	TxR	*Aeromonas*
CH4	Cy5	*Edwardsiella*

Interpretation of results

- A sample is considered valid if the internal control (HEX channel) is detected or if any pathogen target (FAM, TxR, Cy5) is positive, consistent with your internal QC criteria.
- Positive results are indicated by amplification in the channel corresponding to the specific pathogen, above assay defined threshold settings on the instrument.
- The negative control must show no amplification in pathogen channels; any contamination in the negative control invalidates the run and requires repeat testing.

Quality control

Include at least one negative control in every run, and incorporate positive or external controls according to laboratory policy and accreditation requirements. Monitor amplification curves and baseline noise to confirm proper instrument function and assay performance.