KYTO*vial*

PRESERVATION SOLUTION FOR YOUR **MICROBIOME SAMPLES**

- **Long-term preservation** solution for your samples
- **Reduces bio-security risks** during storage and transport
- **Enhances quality** of KYTOS microbiome analyses
- **Small footprint** enables **rapid** and **frequent** sampling

The quality of samples strongly affects the reliability of KYTOS microbiome analyses. Ideally, samples are measured instantaneously, but in practice this is not always possible. Prolonged storage (i.e. > 24h), even at 4 °C, strongly changes the microbial load, and the microbial composition of water samples. Performant sample preservation during storage and transport to the laboratory is therefore of vital importance.

The KYTO*vials* offer a long-term sample preservation system, fine-tuned for a wide range of samples. The vials contain a small amount of a bactericidal component that kills microbial cells and protects their membrane and internal components through a process called *fixation*. This process prepares the samples for direct analysis by flow cytometry by preserving the microbial load and community profile, thereby enabling detailed investigation of the microbial community in the samples.

SPECIFICATIONS

Bactericidal action. Microbial cells are killed by the fixing agent, thereby protecting the user and preventing new microbes from contaminating the sample.

Convenience. KYTO*vials* are supplied in recyclable cardboard boxes.

Small footprint. The vials have a small footprint (i.e. 2 mL total volume) enabling large sampling campaigns and easy storage in the provided storage boxes.

Storage conditions. Unused vials should be stored between 4 - 10 °C for up to 3 months from date of manufacturing in a cool and dry place.

Safety first. Durable materials with a screw-cap washer seal avoid undesirable entry/escape of sample and KytoVial preservation reagents.

Sample identification. Semi-permanent sample identifiers on each vial can resist chemicals, extreme temperatures and extended periods of storage.

PRODUCT PERFORMANCE

STORAGE PERFORMANCE

The storage performance of the KYTO*vial* solution was tested using two types of representative marine aquaculture water samples. These were stored at 4°C and analyzed repeatedly by flow cytometry for up to 1 month of storage. The microbial load in the two types of water samples is stable during storage in KYTO*vials* for up to one month. Sample storage at 4°C without preservation results in significant microbial growth within 4 days for both sample types.

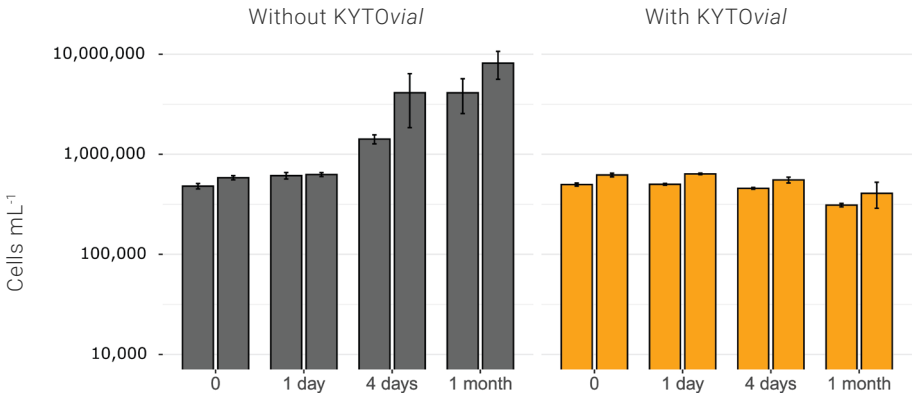


Figure 1: *The cell concentration during one month of storage at 4°C with (orange) and without (grey) the KYTOvial. Traditional sample storage at 4°C results in significant microbial growth within 4 days.*

Preservation of community structure was additionally verified using a fresh water and a marine sample. For each sample, replicated aliquots were stored with and without fixative. Aliquots were stored at 4°C and analyzed repeatedly by flow cytometry for up to 6 months of storage. For both fresh and sea water, community typing revealed that samples without fixative shifted to other community types as compared to before fixation. The community type was preserved well in samples with fixative.

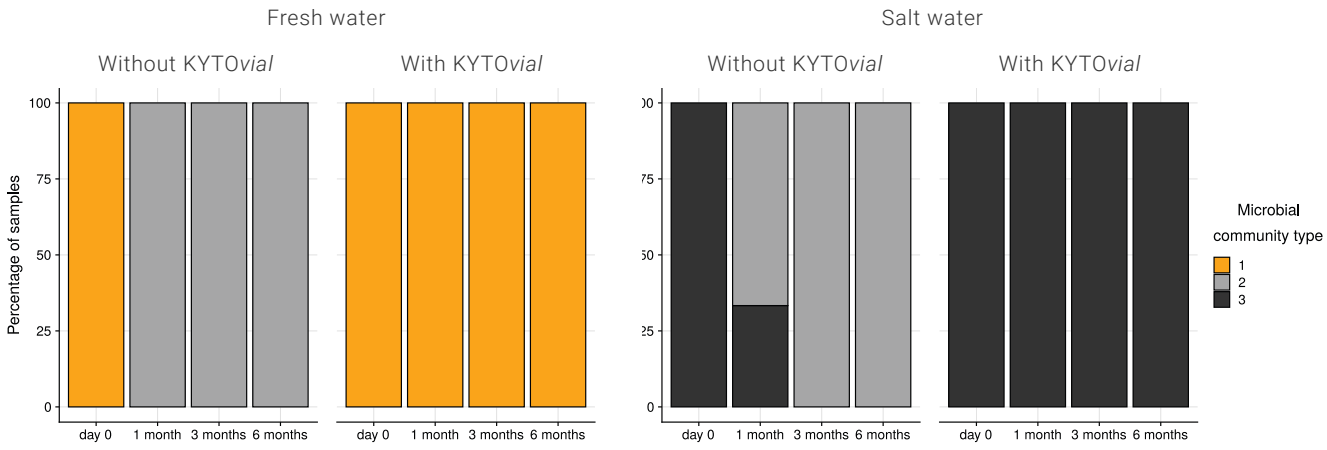
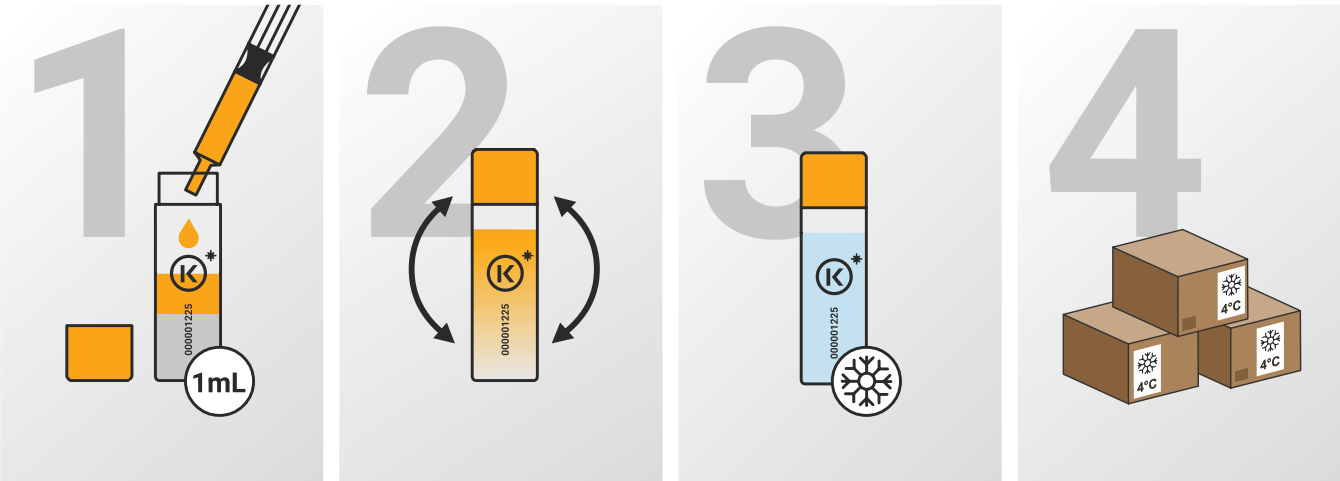


Figure 2: *The community type of sea and fresh water samples stored at 4°C, with and without the KYTOvial fixative solution over a period of six months. Traditional sample storage at 4°C results in shifting to other community types.*

INSTRUCTIONS FOR USE

IMPORTANT: the sampling vials contain small amounts of chemical agents that may present health hazards. Read the provided safety data sheet (SDS) carefully prior to use. Contact your provider if you did not receive the SDS.

WATER SAMPLES



Sampling is executed using a syringe. If these are not available at your facility, request the KYTOS team to provide these with the sampling vials.

1 **No more than 1mL of sample** is transferred into the vial.*

2 Close and **gently shake** the vial.

3 Samples can be stored cooled (4°C - 10°C) for up to **6 months** until analysis.

4 Send samples to a KYTOS lab.

* In case residual peroxide or chlorine is present or expected in the water sample please take the sample from a sodium thiosulfate stabilized water sample. In case of doubt, please contact KYTOS for technical advice.

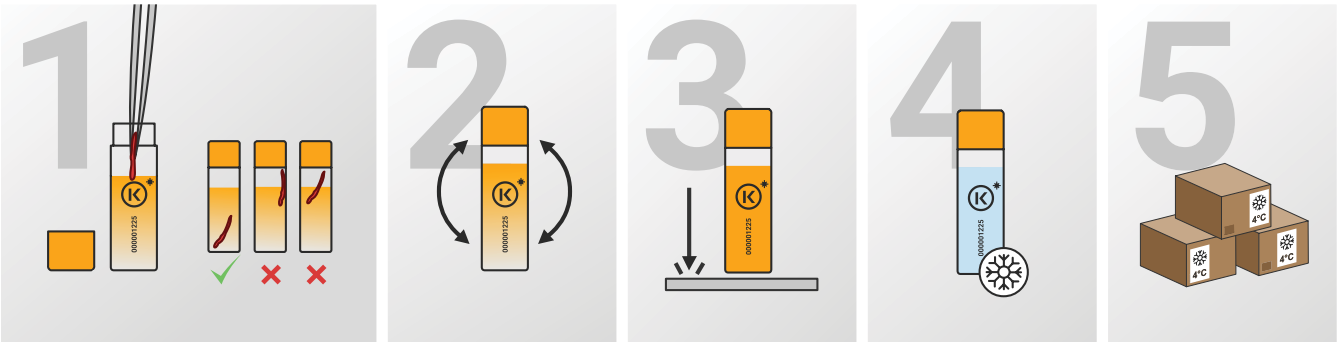
Notes

- There is only a small volume of bactericidal solution in each vial - do not be alarmed if the vials appear empty.
- Contact KYTOS if you are unsure about the sampling process.

SHRIMP GASTRO INTESTINAL (GIT) SAMPLES

Before starting

- Dissect the GIT sample from the **shrimp** using your own protocol.
- We recommend to sample between 0.1 g and 0.5 g of fresh GIT sample for optimal results.
- You do not need to weigh the individual GIT samples as long as you are in between these sample amounts.



Sample prep

1 **Transfer the GIT sample to the Kytovial**

Unscrew the vial cap and place it safely (facing upwards) on the bench. Be careful not to tip over the vial (e.g. by holding it in one hand or having it a sample rack). Work aseptically as much as possible to avoid contamination of samples. Use a fresh and clean forceps, spatula or tweezers to transfer the whole GIT sample into the fixation fluid. **Please make sure the GIT sample is completely submerged** in the fixation liquid (if needed, nudge/fold it in gently with the tweezers, otherwise take a smaller amount of sample). If the same tweezers/spatula is used across multiple samples, cross-contamination may occur which strongly reduces sample quality. Please **use a fresh, clean spatula for every sample**.

2 Close and **gently shake** the vial.

3 Firmly tap the vial on the bench to make sure that the GIT sample is at the bottom of the vial.

4 Samples can be stored cooled (4°C - 10°C) for up to **6 months** until analysis.

5 Send samples to a KYTOS lab.

Notes

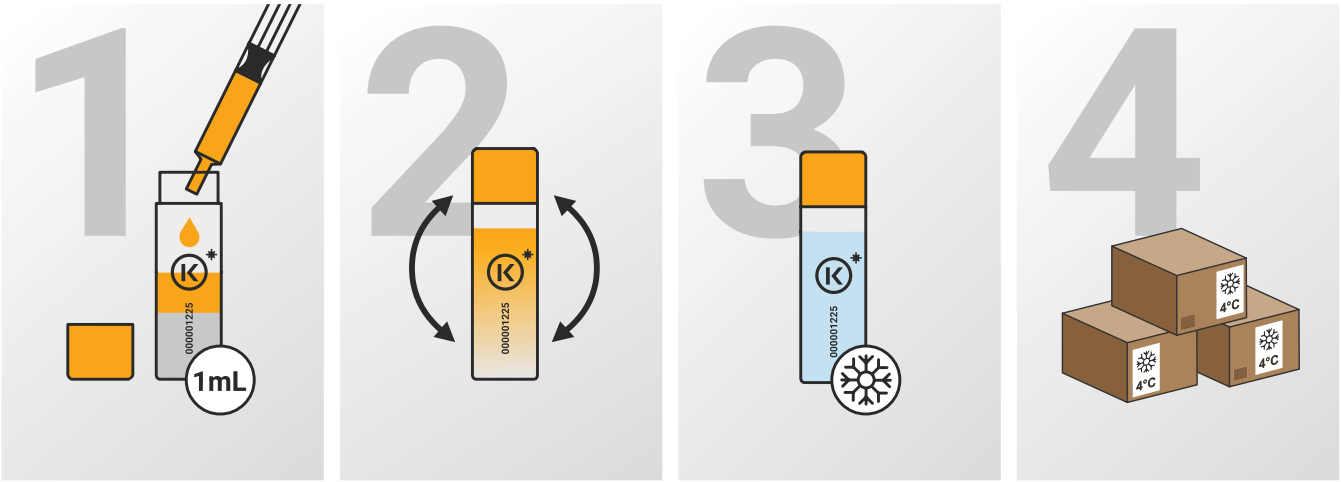
- If the sample cannot be completely submerged into the fixation fluid, or the fluid is “overflowing” after adding the sample, please use a smaller starting amount of GIT sample. The Kytos technology is capable of measuring on relatively small amounts of sample.
- Contact KYTOS if you are unsure about the sampling process.

CONCENTRATED LIVE FEED SAMPLES

Live feeds such as algae and *Artemia* pastes, as well as dry feeds, are concentrated biological matrices. For optimal and reproducible storage and measurement we advise to dilute these samples in a sterile buffer prior to adding them to the KytoVials.

Before starting

- Dilute your sample to watery consistency for easy pipetting.
- If your samples are very dense (e.g. algae paste), contact the KYTOS team for advice on how to sample this matrix best.



Sample prep

Sampling is executed using a syringe. If these are not available at your facility, request the KYTOS team to provide these with the sampling vials.

1 **No more than 1mL of sample** is transferred into the vial.

2 Close and **gently shake** the vial.

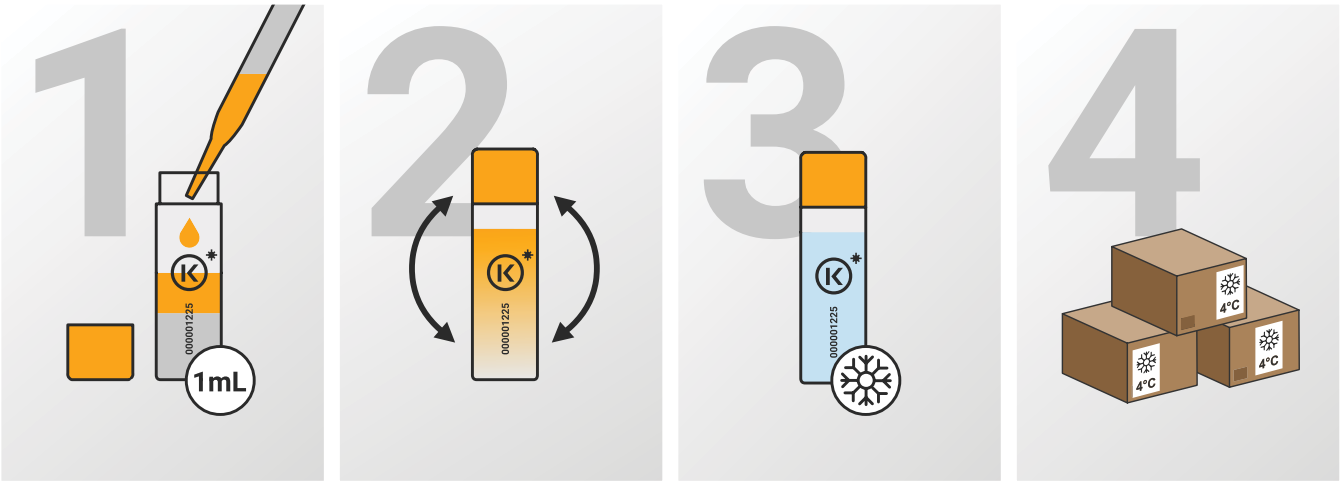
3 Samples can be stored cooled (4°C - 10°C) for up to **6 months** until analysis.

4 Send samples to a KYTOS lab.

Notes

- There is only a small volume of bactericidal solution in each vial - do not be alarmed if the vials appear empty.
- Contact KYTOS if you are unsure about the sampling process.

SEDIMENT AND SLUDGE SAMPLES



Sample prep

1 **Transfer the sediment sample to the Kytovial.**

Unscrew the vial cap and place it safely (facing upwards) on the bench. Be careful not to tip over the vial (e.g. by holding it in one hand or having it in a sample rack). Work aseptically as much as possible to avoid contamination of samples. Use the pipette provided with the sampling kit to transfer approximately 0.5 mL of sediment in the fixation liquid. If the same pipette is used across multiple samples, cross-contamination will occur which strongly reduces sample quality. **Please use a new pipette for every sample.**

2 Close and **gently shake** the vial.

3 Samples can be stored cooled (4°C - 10°C) for up to **6 months** until analysis.

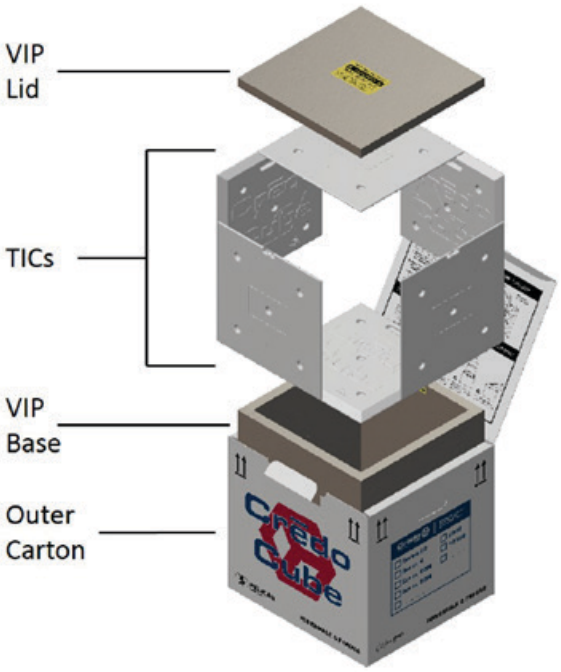
4 Send samples to a KYTOS lab.

Notes

- Contact KYTOS if you are unsure about the sampling process.

USER GUIDE OF CRĒDO CUBE™ PARCEL SHIPPERS

Receiving the Crēdo Cube™



1 Open the lid carefully.

2 Take of the top VIP lid. Take care that the plastic film is not broken, so the vacuum stays intact.

3 Take of the top TIC (Can be cold !!!).

4 Take out the vials/samples delivered.

5 Take out the TIC's and let them thaw at room T°.

Cleaning of the Cube and TIC's

1 Clean the TIC's with warm water and soap or alcohol. Decontaminate with 70% isopropanol

2 Clean the insulator cube with a damp towel with soap.

3 Clean the plastic outer box with some 70% isopropanol.

Packing samples for shipment

Conditioning the TIC's

1 Put the TIC's in a flat position at -18°C or lower for at least 24h.

2 Check if fully frozen by shaking the TIC. If fluid is still detected, keep in the freeze until fully frozen.

3 Before packing the samples place the TIC's at room T° for 35 min, this staging time will help the TIC's get to the correct temperature.

4 The expected transport temperature should be between 3 - 4,5°C.

IMPORTANT: RESPECT 35 MIN CONDITIONING TIME OF TIC'S BEFORE PACKING

Packing the cubes

1. Insert the insulator box inside the plastic outer box.
2. Insert a TIC panel into the insulator base with the Crēdo Cube logo facing up.
3. Add the 4 TIC panels to form the side walls with the Crēdo Cube logo facing in.

Insert the payload

1. Make sure the samples are pre-conditioned at around 5°C.
2. Insert the samples between the TIC's.
3. Fill the empty spaces with a non-insulating filler.

Close the cube

1. Put on the final TIC with the Crēdo Cube logo facing in. Make sure there is **no air gap between the TIC's**.
2. Put on the top of the insulator box. **The warning sticker should be facing up. No air gap should be observed.**

3. Close and secure the plastic box before shipping.

References

1. UG-001-EN-E Peli Crēdo Cube Series 4 User Guide - Rev E © Peli BioThermal 2020