

Guidance for sampling and shipping of samples for real time RT-PCR

Recommended equipment

- Ovarian fluid/milt: barcode tube **containing RLT-buffer**
- Fry/tissue: barcode tube **containing RNAlater**
- Solid envelope
- Sterile forceps and scalpels
- Gas burners for sterilization of tweezers and scalpels
- Clean surface for cutting of tissue samples

The sampling package with barcode tubes from PHARMAQ Analytiq contains a form for ordering analysis. We recommend that you fill in the form as the samples are being taken.

We encourage to register all samples electronically before shipment.

Preferred tissue

Virus	Tissue	Bacteria	Tissue	Parasites	Tissue
Piscine Myocarditis virus (PMCV)	Heart, Ovarian fluid, Milt	<i>Flavobacterium psychrophilum</i>	Kidney, Gill	Paramoeba perurans	Gill
Piscine Orthoreovirus (PRV)	Heart, Ovarian fluid, Milt	<i>Yersinia ruckeri</i>	Kidney	Paranucleospora theridion	Gill
Salmon Gill Poxvirus (SGPV)	Gill	<i>Renibacterium salmoninarum</i>	Kidney, Ovarian fluid, Milt	Parvicapsula pseduobranchicola	Gill
Infectious Pancreas Necrosis virus (IPNV)	Kidney, Ovarian fluid, Milt	<i>Piscirickettsia salmonis</i>	Kidney	Ichtyobodo sp. *(Costia)	Gill
Pancreas Disease virus (SAV)	Heart	<i>Branchiomonas cysticola</i>	Gill	Nucleospora cyclopteri	Gill, Kidney
Infectious Salmon Anaemia virus (ISAV)	Heart, Ovarian fluid, Milt	<i>Clavochlamydia salmonicola</i>	Gill		
Viral Haemorrhagic Septicaemia virus (VHSV)	Kidney	<i>Moritella viscosa*</i>	Wound, Kidney		
Nodavirus (VNN)	Kidney, CNS, Ovarian fluid, Milt	<i>Pasteurella sp.</i>	Kidney		
Infectious hematopoietic necrosis virus (IHNV)	Kidney	<i>Aeromonas salmonicida*</i>	Kidney		
Atlantic Halibut Reovirus	Liver, Kidney	<i>Tenacibaculum sp.</i>	Wound, Kidney		
Lumpfish Flavi Virus	Kidney / Liver, Ovarian fluid, Milt	<i>Tenacibaculum maritimum</i>	Wound, Kidney, Gill		
Cyclopterus lumpus Coronavirus (CluCV)	Kidney, Ovarian fluid, Milt	Trippel analysis for <i>Vibrio anguillarum</i> - analysis for O1, O2α and universal (all known variants of the bacteria)	Kidney		
Cyclopterus lumpus Totivirus (CluTV)	Kidney, Ovarian fluid, Milt	<i>Vibrio anguillarum O1</i>	Kidney		
		<i>Vibrio anguillarum O2α</i>	Kidney		
		<i>Francisella philomiragia ssp. noatuensis</i>	Kidney		

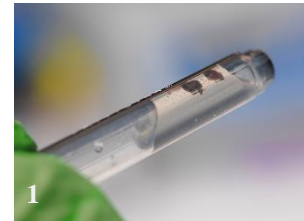
*Subtyping possible

Guidance for sampling

Sampling for RT-PCR should be performed using a sterile technique to ensure that contamination is avoided.

- Use paper to remove residues of tissue from the scalpel
- Use a gas burner to sterilize the scalpel blade and tweezers between each fish
- You may use the sterile interior of the scalpel blade packaging as a surface upon which to trim tissue
- You should avoid contact between head kidney/heart tissue and the abdominal cavity

The tissue samples should be the size of a match head, 2x2x2mm. It is important that the tissue samples are properly immersed in the fluid contained in the sample tubes (1).



LARVAE:
JUVENILE:

Place the whole larva in a tube with RNAlater
Remove the head just behind the operculum, the remaining juvenile may be split in two before being placed in a tube with RNAlater. It is important to include gills, heart and kidney from each fish.

SMOLT/SEA WATER FISH:

01. Gill (arch number 2) and skin should be sampled first (2A, 2B and 2C)
02. Place the tissue on a clean area and split each sample in two pieces of similar size. (A- and B sample) Put both samples (of the same organ) into a tube containing RNAlater



03. Sterilize the scalpel blade and tweezers before making a cut in the heart cavity. Use the tweezers to pick up the heart by the bulbus area (3)
04. Place the heart on a clean area and cut off the apex, split the apex in two and put both samples in a tube containing RNAlater
05. Any residues of tissue should be removed from the instruments which should then be sterilized before making a cut in the abdominal cavity (4)
06. The kidney should be the first organ sampled from the abdominal cavity. Identify the kidney by removing the swim bladder. Sterilize the instruments again before extracting a small square from the head kidney. Split the extracted square in two and put both samples in a tube containing RNAlater (5)



07. If necessary sample the remaining organs from the abdominal cavity, sterilizing the instruments in between organs



08. When sampling is finished the order form should be completed.

09. Make a note on the barcode sheet if additional information regarding the tubes (i.e. cage, wound, healthy) needs to be included

10. Place the sample tubes in a strong envelope together with order form, barcode sheet, and a cooling element. The samples should be sent using express delivery. (6)

11. For storing of samples over a longer period before submission the samples should be stored overnight in fridge to ensure proper fixation. Then the samples should be stored in freezer.

Guidance for sampling of brood fish

Sampling for RT-PCR should be performed using a sterile technique to ensure that contamination is avoided.

OVARIAN FLUID/MILT: Minimum 0,2 ml and maximum 1 ml ovarian fluid/milt in a tube containing RLT-buffer

If the samples are sent within 24 hours the tubes can be stored in the fridge. If the samples are stored for longer than this it should be done at -20C. It is important to ensure proper cooling during transportation!

If anything is unclear – please contact us!

Ship the samples with express delivery:

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