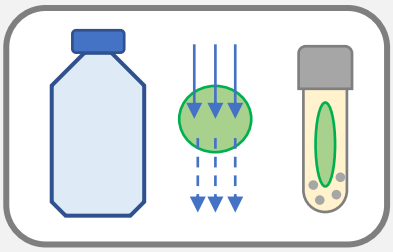
CARLA KIT (microalgae) – Short Protocol

To generate technical duplicates from one sampling point.

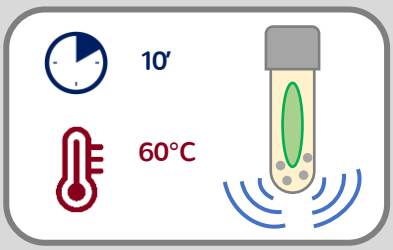
Sample preparation

1. WATER COLLECTION-FILTRATION SAMPLE CONSERVATION



- Collect 1L of water.
- Filter the water. Transfer immediately the filter in a tube with beads, add 1 mL of RNA Lysis buffer.

2. CELL LYSIS

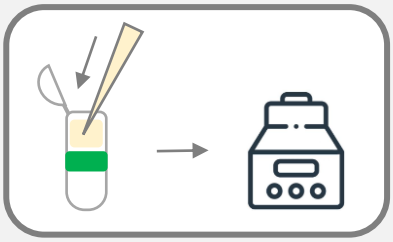


- Place at 60°C for 10 min.
- Vortex/bead beating for 2 min.

3. RNA EXTRACTION

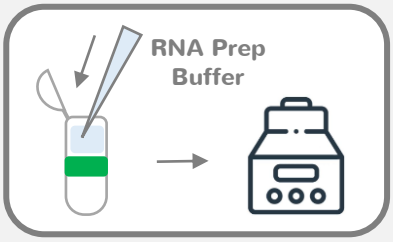
Perform all steps at RT and centrifugation at >10,000 g for 30 s, unless specified.

- Centrifuge, collect the supernatant, transfer in a new tube.
- Add 1 mL of ethanol 95-100% [1:1]$_{Vol}$, mix well.

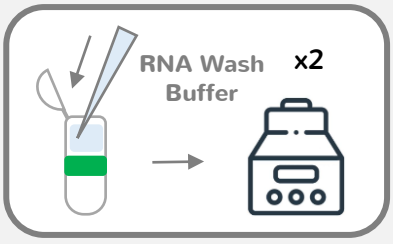


- Transfer in a green column, centrifuge.
- Discard the flow-through.

------- 3 rounds needed -------



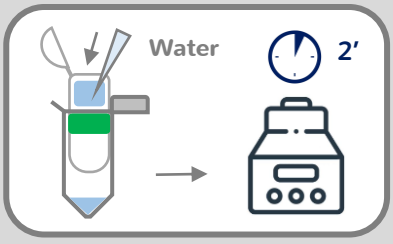
- Add 400 µL RNA Prep Buffer, centrifuge.
- Discard the flow-through.



- Add 700 µL RNA Wash Buffer, centrifuge. Discard the flow-through.
- Add 400 µL RNA Wash Buffer, centrifuge for 1 min for complete removal of the buffer.

4. RNA ELUTION

Measure RNA concentration with a spectrophotometer (absorbance at 260 nm) – optional.



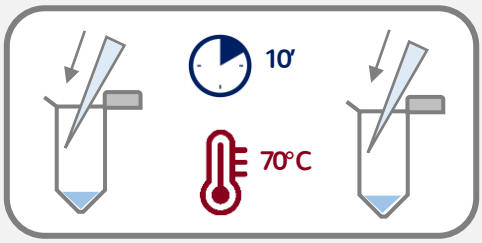
- Transfer the column into a new tube.
- Add 100 µL Mol.Biol.Water, centrifuge for 2 min.

CARLA KIT (microalgae) – Short Protocol

Before Starting: Dilute Solution A and Wash buffer. Prepare microplate strips.
All solutions should be at RT prior to use. Mix gently and spin down before use.

Protocol A

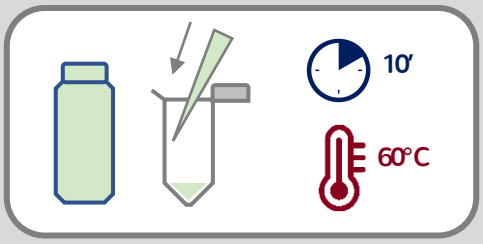
1. RNA FRAGMENTATION



- Add 4 µL of Fragmentation buffer to each 100-µl RNA eluate.
- Mix gently, place at 70°C for 10 min.
- Add 4 µL of STOP Fragmentation solution.

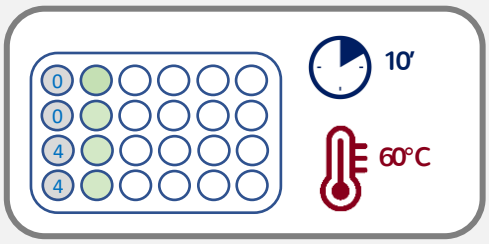
2. PRE-HYBRIDIZATION

VOL pre-hybridization mix = (70 µL Hybr buffer + 30 µL water)
X number of samples



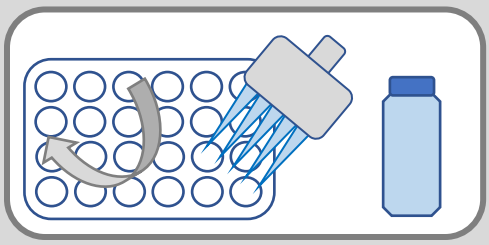
- Add 100 µL of Pre-hybridization mix.
- Mix well and place at 60°C for 10 min.

3. TRANSFER TO MICROPLATE - HYBRIDIZATION



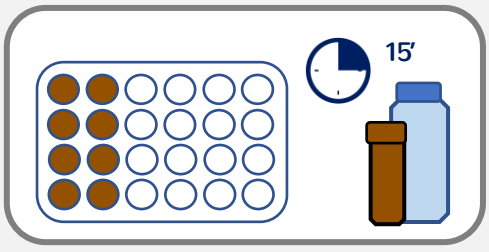
- Fill the first wells with 100 µL of STD0 (NC) and STD4 (PC), in duplicates.
- Continue with duplicates of pre-hybridized samples (100 µL per well).
- Cover and incubate at 60°C for 10 min.

4. WASHING



- Discard the liquid from the wells and wash with Wash buffer 3 times.

5. ANTIBODY ADDITION

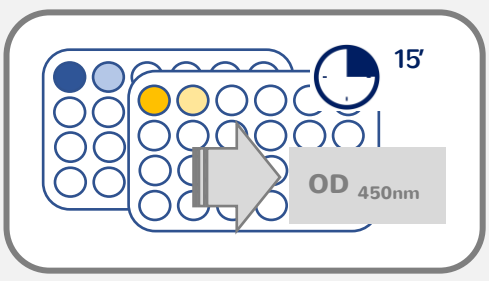


- Add 100 µL of Solution A (dark).
- Incubate at RT for 15 min.

6. WASHING

Repeat washing as in 4.

7. COLORIMETRIC DETECTION



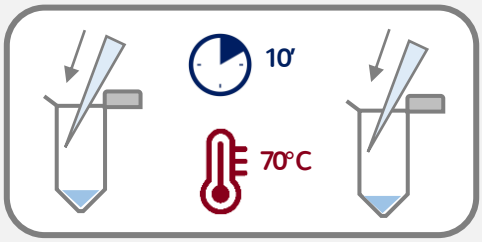
- Add 100 µL of Solution B, incubate at RT for 15 min (dark).
- Add 50 µL of STOP Solution B and measure the absorbance (OD_{450} nm).

CARLA KIT (microalgae) – Short Protocol

Before Starting: Dilute Solution A and Wash buffer. Prepare microplate strips and remove liquid.
All solutions should be at RT prior to use. Mix gently and spin down before use.

Protocol B

1. RNA FRAGMENTATION

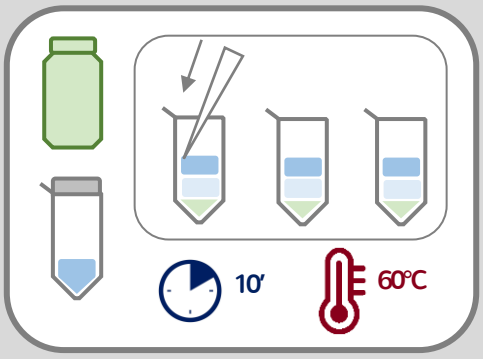


- Add 4 µL of Fragmentation buffer to each 100-µl RNA eluate.
- Mix gently, place at 70°C for 10 min.
- Add 4 µL of STOP Fragmentation solution.

2. DILUTIONS AND PRE-HYBRIDIZATION

HB = Hybridization buffer

Resulting dilutions :
Tube 1 – 1/2
Tube 2 – 1/4
Tube 3 – 1/10

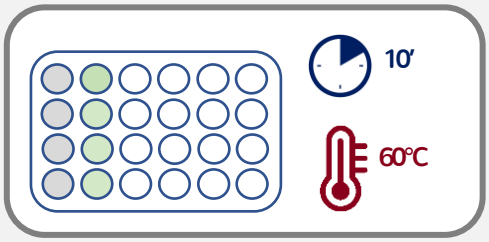


- Prepare 3 new tubes. Fill each tube in the following sequential order:

Tube 1
70 µL HB + 80 µL water + 50 µL RNA eluate
Tube 2
70 µL HB + 105 µL water + 25 µL RNA eluate
Tube 3
70 µL HB + 120 µL water + 10 µL RNA eluate

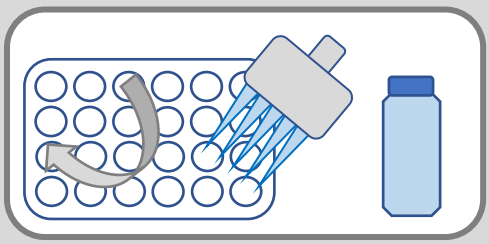
- Mix well and place at 60°C for 10 min.

3. TRANSFER TO MICROPLATE - HYBRIDIZATION



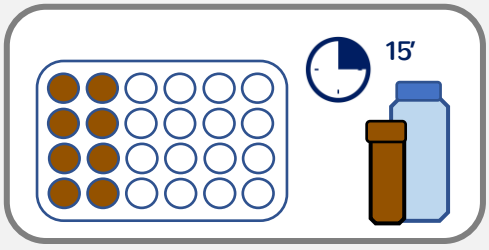
- Fill the first wells with 100 µL of each standard solution (STD 0, 1, 2, 3 and 4), in duplicates.
- Continue with duplicates of pre-hybridized sub-samples (100 µL / well) .
- Cover and incubate at 60°C for 10 min.

4. WASHING



- Discard the liquid from the wells and wash with Wash buffer 3 times.

5. ANTIBODY ADDITION

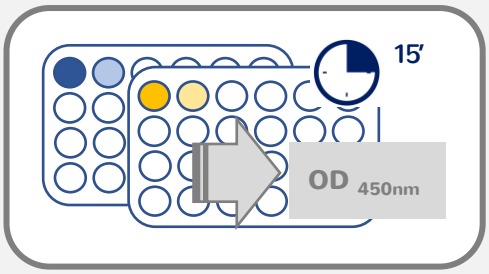


- Add 100 µL of Solution A (dark).
- Incubate at RT for 15 min

6. WASHING

Repeat washing as in 5.

7. COLORIMETRIC DETECTION



- Add 100 µL of Solution B, incubate at RT for 15 min (dark).
- Add 50 µL of STOP Solution B and measure the absorbance (OD_{450} nm).