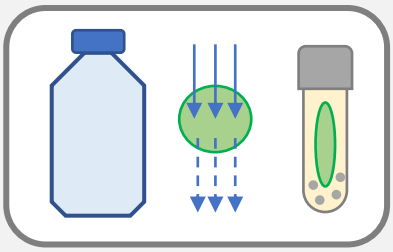
CARLA KIT (cyanobacteria) – Short Protocol

To generate technical duplicates from one sampling point.

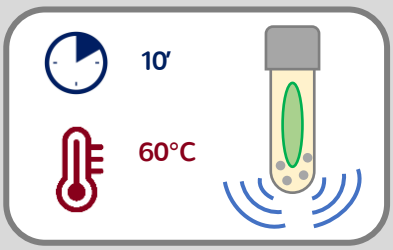
Sample preparation

1. WATER COLLECTION-FILTRATION SAMPLE CONSERVATION



- Collect **20 mL** of water.
- Filter the water. Transfer immediately the filter in a tube with beads, add **1 mL** of RNA Lysis buffer.

2. CELL LYSIS

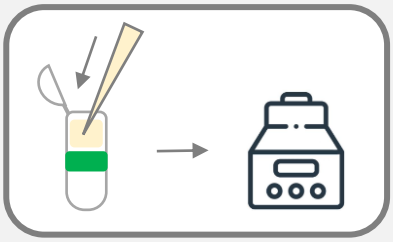


- Place at 60°C for 10 min.
- Vortex/bead beating for 2 min.

3. RNA EXTRACTION

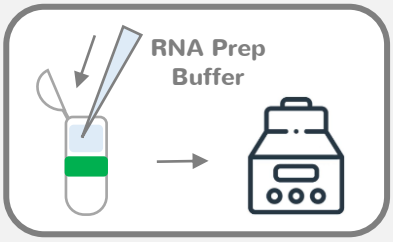
Perform all steps at RT and centrifugation at >10,000 g for 30 s, unless specified.

- Centrifuge, collect the supernatant, transfer in a new tube.
- Add **1 mL** of ethanol 95-100% [1:1]VOL, mix well.

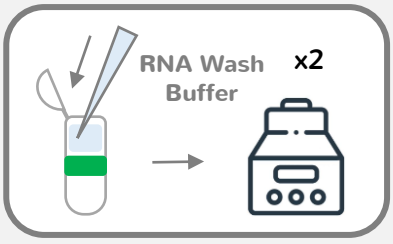


- Transfer in a green column, centrifuge.
- Discard the flow-through.

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



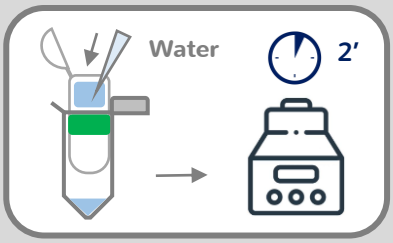
- Add **400 µL** RNA Prep Buffer, centrifuge.
- Discard the flow-through.



- 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.



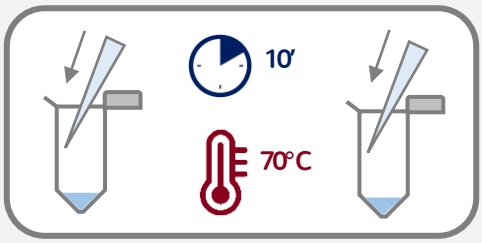
- Transfer the column into a new tube.
- Add **100 µL** Mol. Biol. Water, centrifuge for 2 min.

CARLA KIT (cyanobacteria) – 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

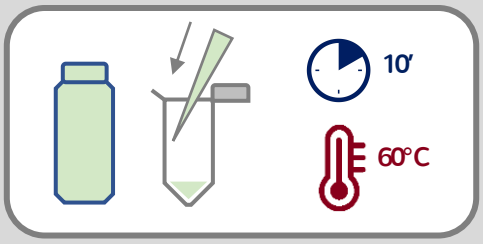
1. RNA FRAGMENTATION



- 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.

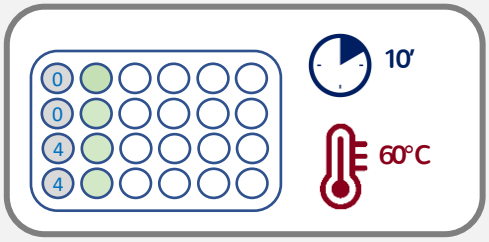
2. PRE-HYBRIDIZATION

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



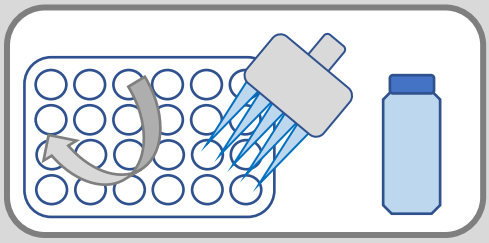
- Add **100 µL** of Pre-hybridization mix.
- Mix well and place at 60°C for 10 min.

3. TRANSFER TO MICROPLATE - HYBRIDIZATION



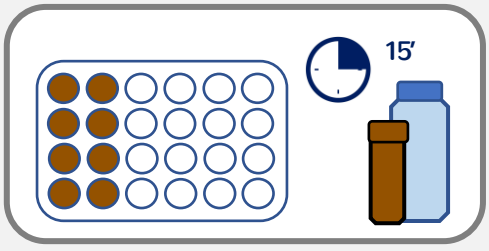
- Fill the first wells with **100 µL** of STD0 (NC) and STD4 (PC), in duplicates.
- Continue with **100 µL** of pre-hybridized samples (duplication by using 2 wells).
- Cover and incubate at 60°C for 10 min.

4. WASHING



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

5. ANTIBODY ADDITION

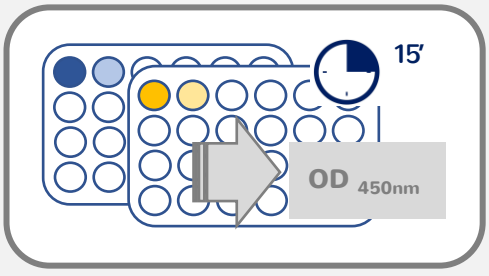


- Add **100 µL** of Solution A (dark).
- Incubate at RT for 15 min.

6. WASHING

Repeat washing as in 4.

7. COLORIMETRIC DETECTION



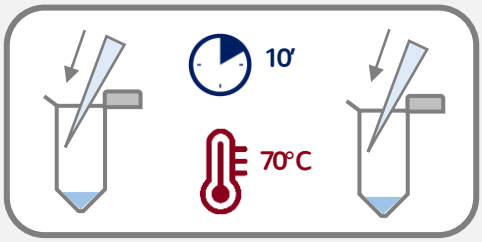
- Add **100 µL** of Solution B, incubate at RT for 15 min (dark).
- Add **50 µL** of STOP Solution B and measure the absorbance (OD_{450} nm).

CARLA KIT (cyanobacteria) – 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 B

1. RNA FRAGMENTATION



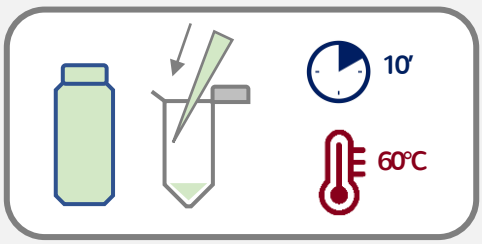
- Add **4 µL** of Fragmentation buffer to each 100-µl RNA eluate.
- Mix gently, place at 70°C for 10 min.
- Add **4 µL** of STOP Fragmentation solution.

2. DILUTION

Proceed with two dilutions series in **100 µL** (1/10 and 1/100)
→ keep 3 sub-samples of **90 µL**

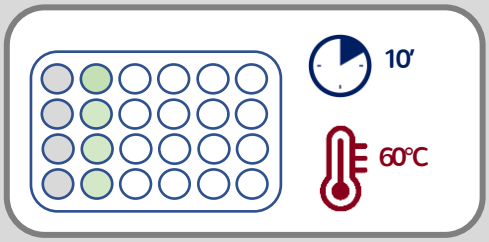
3. PRE-HYBRIDIZATION

VOL pre-hybridization mix = (70 µL Hybr buffer + 40 µL water)
X number of samples
X3 -dilutions-



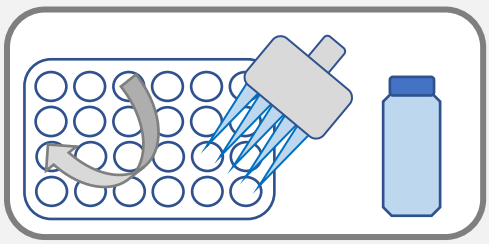
- Add **110 µL** of Pre-hybridization mix.
- Mix well and place at 60°C for 10 min.

4. TRANSFER TO MICROPLATE - HYBRIDIZATION



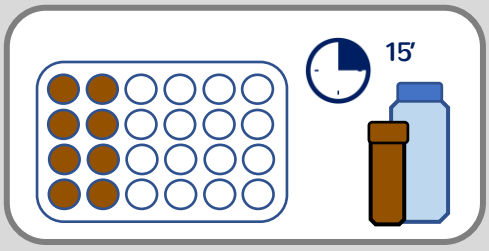
- Fill the first wells with **100 µL** of each standard solution (STD 0, 1, 2, 3 and 4), in duplicates.
- Continue with **100 µL** of pre-hybridized sub-samples (duplication by using 2 wells).
- Cover and incubate at 60°C for 10 min.

5. WASHING



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

6. ANTIBODY ADDITION

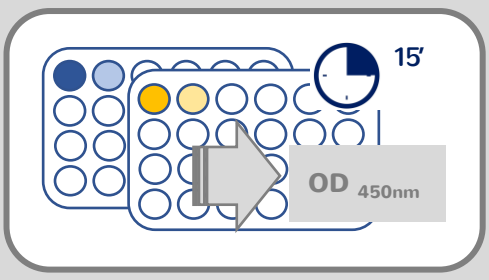


- Add **100 µL** of Solution A (dark).
- Incubate at RT for 15 min

7. WASHING

Repeat washing as in 5.

8. 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).