Feb 14, 2022

RNA/DNA extraction from plankton natural samples using NucleoSpin RNA + RNA/DNA Buffer kits (Macherey Nagel)

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dx.doi.org/10.17504/protocols.io.b2j7qcrn

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This protocol has been developped for simultaneous nucleic acid (RNA and DNA) extraction from environmental water samples collected by filtration on polycarbonate 47mm and 142mm filters.

It has been developped during BioMarks project (2009-2013) and has been used in many projects studying plankton diversity (TARA-OCEANS 2009-2013, TARA-PACIFIC 2016-2018, TONGA 2019), and plankton monitoring projects (MOOSE-GE 2017-ongoing).

This protocol is applied in routine in our lab and also in CEA Genoscope.

DOI

dx.doi.org/10.17504/protocols.io.b2j7qcrn

Sarah Romac 2022. RNA/DNA extraction from plankton natural samples using NucleoSpin RNA + RNA/DNA Buffer kits (Macherey Nagel). **protocols.io** https://dx.doi.org/10.17504/protocols.io.b2j7qcrn

protocol

Alberti A., Poulain J., Engelen S., Labadie K., Sarah Romac S., [...], Wincker P. Viral to metazoan marine plankton nucleotide sequences from the TaraOceans expedition. Scientific Data (2017). dx.doi.org/10.17504/protocols.io.qv6dw9e

protocol ,

Dec 03, 2021

Feb 14, 2022

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This protocol has been modified from 3 references provided by Macherey-Nagel :

- **NucleoSpin RNA Mini kit** (Macherey-Nagel, ref 740955.50) (50 preps) ;

- **NucleoSpin RNA Midi kit** (Macherey-Nagel, ref 740962.20) (20 preps) ;

Instruction-NucleoSpin-RNA.pdf

- **NucleoSpin® RNA/DNA Buffer Set** (Macherey-Nagel, ref 740944) (100 preps).

Instruction-NucleoSpin-RNA-DNA-Buffer-Set.pdf

Kits and reagents :

- **NucleoSpin RNA Mini kit** (Macherey-Nagel, ref 740955.50) (50 preps) :
  -Store lyophilized RDNase A at +4°C on arrival;
  -Store all other kits components at room-temperature.

- **NucleoSpin RNA Midi kit** (Macherey-Nagel, ref 740962.20) (20 preps) :
  -Store lyophilized RDNase A at +4°C on arrival;
  -Store all other kits components at room-temperature.

- **NucleoSpin® RNA/DNA Buffer Set** (Macherey-Nagel, ref 740944) (100 preps).

- **β-mercaptoethanol** (Sigma-Aldrich, ref 63689-25ML-F).

Specific equipments :

- Chemical hood Captair MID CAP 633 (Erlab)
- Centrifuge 5417R equipped with rotor 30 wells 1.5-2mL microtubes (Eppendorf)
- Centrifuge 5804R equipped with Swing Rotor 16 wells Falcon 15mL tubes (Eppendorf)
- Thermomixer equipped with Thermoblock 24 tubes 1.5mL (Eppendorf)

Always wear a labcoat, and clean gloves for each step.

Decontaminate all the area (chemical hood, centrifuges, thermomixer, pipettes, and racks) with RNase away before to start.

Always use filter tips and sterile 1.5mL microtubes.

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Work under a chemical hood.

- **rDNase**: add the volume of RNase-free H2O recommended from each kit, incubate 1min at Room Temperature.
Don't vortex!
Do aliquots of 150µL and store at -20°C. Do not freeze/thaw the aliquots more than 3 times.

- **Wash Buffer RA3**: add 50ml EtOH 96-100%.
- **DNA Wash Buffer**: add 90ml EtOH 50%.
- Cool centrifuge to 19°C
- Warm up **DNA Elute Buffer** and **Nuclease-free** water to 50°C.
- Check if clean cisors (DNA/RNA free) are available.
- Heat the Thermomixer (Eppendorf) to 50°C for DNA elution.

Lysis 6m

1 Lyse your plankton filter samples following the protocol :

https://www.protocols.io/edit/cryogrinding-protocol-mecanic-lysis-of-142mm-filte-beqpjdvn

2 Depending of the plankton size fraction samples you have, select :

1 - RNA/DNA extraction using NucleoSpin RNA MIDI kit regarding [>20µ] size fractions (samples collected and prefiltered using a plankton net) ;

2 - RNA/DNA extraction using NucleoSpin RNA MIDI and MINI kits regarding [<20µ] size fractions (samples collected using NIskin bottles or pumping and filtered on 142mm filters) ;

3 - RNA/DNA extraction using NucleoSpin RNA MINI kits regarding [<20µ] size fractions (samples collected using NIskin bottles or pumping and filtered on 47mm filters) ;

Step 2 includes a Step case.
1 _ [>20] - 47mm - NucleoSpin RNA MIDI
2 _ [<20] - 142mm - NucleoSpin RNA MIDI and MINI
3 _ [<20] - 47mm - NucleoSpin RNA MINI

step case

1 _ [>20] - 47mm - NucleoSpin RNA MIDI

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1 - RNA/DNA extraction using NucleoSpin RNA MIDI kit regarding [>20µ] size fractions (samples collected and prefiltered using a plankton net).

3 In the Falcon50 tube containing the filter powder, add **3.6 mL of RA1 and 36 µL of β-mercaptoethanol** per sample. Vortex thoroughly. 5m

Incubate at room temperature for about 5 minutes.

00:05:00 **Room temperature**

4 Transfer the lysate and the filter on a Nucleospin Filter placed in a Collection Tube 15 mL. **(from NucleoSpin RNA MIDI kit)** 10m

Centrifuge 10 min at 4500g at 19°C

4500 x g, 19°C, 00:10:00

5 Discard the Nucleospin Filter.

Transfer the eluate in a new sterile Falcon15mL tube.

Volume is around **3.7 mL** .

Precipitation / Purification 6m

6 Add **4 mL EtOH 70%** to the filtrate and and **vortex 2 x 5 sec** to mix.

Volume is around **7.7 mL** .

7 Transfer **3 mL from the lysate/ethanol-mix** in a **NucleoSpin RNA MIDI column** placed in a Collection Tube 15mL. **(from NucleoSpin RNA MIDI kit)** 3m

Centrifuge 3min at 4500g at 19°C.

4500 x g, 19°C, 00:03:00

Discard flow through and replace the NucleoSpin RNA MIDI column on the Collection Tube.

8 Repeat Step 7 until all the volume **lysate/ethanol-mix** have been passed through the NucleoSpin RNAMDI column.

DNA Purification and Elution 6m

9 Add **2.5mL DNA Wash** to the NucleoSpin RNA MDI column. 3m

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Centrifuge 3min at 4500g at 19°C.
4500 x g, 19°C, 00:03:00

Discard the flow-through and reuse Collection Tube;

10 Add again **2.5mL DNA Wash** to the NucleoSpin RNA column. 3m

Centrifuge 3min at 4500g at 19°C.
4500 x g, 19°C, 00:03:00

Discard the flow-through and place the Column in a new sterile Falcon15mL.

11 Let dry the column for 5min at room temperature. 5m
00:05:00 **Room temperature**

12 Add **300µL DNA Elute** (preheated to **50°C**) directly onto the membrane and incubate 5 min at room temperature. 8m
00:05:00 **Room temperature**

Centrifuge 3min at 11000g;
11000 x g, 19°C, 00:03:00

Place the NucleoSpin RNA MIDI column into ta new 15mL Collection tube for the following DNA digestion (see Step 14).

13 Transfer the DNA extract on a sterile 1.5mL sterile microtube correctly annotated (Sample code, DNA extraction date, operator).

Store DNA extracts at -20°C.

DNA Digestion 6m

14 **Prepare DNase mix**: 235µL Reaction Buffer for DNase + 25µL reconstituted rDNase per sample and mix gently.
- for 10 samples: 2350µl + 250µl ;
- for 17 samples: 3995µl + 425µl ;

Apply **235µL DNase Mix** directly onto the center of the column and incubate 15 min at room temperature.
00:15:00 **Room temperature**

DNAase Inactivation and RNA purificationn 6m

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15 Add **2.6mL RAW2** (inactivation of the rDNase).
Incubate at room temperature for 1min. 3m

Centrifuge 3min at 4500g.
4500 x g, 19°C, 00:03:00

Place the Column into a new Collection Tube.

16 Centrifuge 3minmin at 454500g. 3m
4500 x g, 19°C, 00:03:00

PlaPce the ColCmn into a na newCollection Tube.

17 Add **2.6mL RA3.** 5m

Centrifuge 5min at 4500g.
4500 x g, 19°C, 00:05:00

Discard flow-through and place the NucleoSpin RNA MIDI column into ta new Falcon 15mL tube.

RNA Elution 6m

18 Add **300µL RNase-free water** (preheated to 50°C) directly onto the membrane . 5m

Incubate 2min at room temperature.
00:02:00 **Room temperature**

Centrifuge 3min at 4500g.
4500 x g, 19°C, 00:03:00

19 Repeat step 18 by eluting with the same eluate.

Discard the NucleoSpin RNA MIDI column.

20 Transfer the RNA extract on a sterile 1.5mL sterile microtube correctly annotated (Sample code, RNA extraction date, operator).

Store RNA extract at -80°C.

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