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eRNA and eDNA co-extraction from water samples filtered through 47mm diameter filters (NucleoMag DNA/RNA Water Kit - MACHEREY NAGEL).

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We use this protocol and it's working

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Abstract

The objective of this protocol is the **environmental RNA (eRNA) and DNA (eDNA) co-extraction from water samples filtered through 47 mm diameter filters.**

RNA extraction is performed using a **MagnetaPure 32 Nucleic Acid Purification System** (Dutscher) and with the **NucleoMagDNA/RNA Water Kit** (Macherey Nagel), with a rDNase digestion step.
DNA extraction is performed using a **MagnetaPure 32 Nucleic Acid Purification System** (Dutscher) and with the **NucleoMagDNA/RNA Water Kit** (Macherey Nagel).

The procedure is based on **reversible adsorption of nucleic acids to paramagnetic beads** under appropriate buffer conditions.

The benefits of using the **MagnetaPure 32 Nucleic Acid Purification System** (Dutscher) are **increased productivity and repeatability, as well as eliminating human error and the pain of repetitive work**.

This protocol is used **prior to molecular biology analysis** (e.g. qPCR, metabarcoding, ddPCR) to specifically target **both macro- and micro-organisms eRNA and eDNA extracted from water samples.**

This protocol is optimised for rare eDNA/RNA and the suggested **elution volumes are therefore low** (between 50 and 65 µL), but can be increased if targets are more abundant.

Image Attribution

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Guidelines

The main steps of the protocol are:

- Material and rDNase preparation
- Plate preparation
- Sample lysis
- eRNA/eDNA co-extraction with the MagnetaPure 32 System (Dutscher)
- eRNA/eDNA elution

Materials

- **Materials:**

- 1000 µL pipette
- 100 µL pipette
- 10 µL pipette
- Scissors
- Vortex + benchtop centrifuge for 5 mL tubes
- Horizontal vortex with 5 mL tube holder (15 mL tube holders)
- Centrifuge for 2 mL tubes (relative centrifugal force needed: 11,000 x g)
- MagnetaPure 32 Nucleic Acid Purification System (Dutscher)
- Specific DNA/RNA-workstation (sterile area equipped with air filtration and UV systems)

- **Consumables:**

All tubes and tips must be sterile and open under the workstation

- 1000 µL tips with filter
- 100 µL tips with filter
- 10 µL tips with filter
- 50 mL tubes: 4 to prepare aliquots, 3 for scissors decontamination
- 5 mL tubes: 1 per 8 samples to prepare NucleoMag B-Beads and MWA2 mix, 1 for rDNase dilution
- 2 mL tubes: 1 per sample to transfer lysate + 2 to prepare aliquots
- 0.5 mL tubes: 3 per sample to transfer eluted RNA
- 96-well plate with 2 mL deep-wells, U-Bottom (Macherey Nagel - 746032.DEEP): 1 per 16 samples RNA and 1 per 16 samples DNA
- Magnetic rod coverfor MagnetaPure 32 (Macherey Nagel – 747032.TC): 1 per 8 samples
- 2 Plastics film to protect the 96-well plate
- Gloves

For any manipulation in a rare DNA/RNA room, provide complete equipment (disposable coat, cap, mask, shoe covers & gloves).

- **Reagents:**

- rDNAse Set (Macherey Nagel - 740963)
- Reaction Buffer for rDNase (Macherey Nagel - 740834.60)
- NucleoMag DNA/RNA Water kit (Macherey Nagel)

Note: shelf life of reagents for 24 months from production

- To clean the scissors:
  - Solution to remove RNA (e.g.*RNase-off*/DNA-off, *RNA/DNA away*)

- H2O2 10 %
- Ethanol 96% for molecular biology
- Molecular biology grade water

- **Samples to be extracted:**

- Filters in 5 mL tubes and stored at -80°C until RNA/DNA extraction

Troubleshooting

Before start

- **Filtration and preservation of water samples through 47 mm diameter filters.**

- **As eRNA degrades very rapidly, the water samples must be filtered and preserved very quickly after sampling** (less than one hour if possible). If filtration is carried out in the field, the filters should be immediately frozen in liquid nitrogen and then stored at -80°C until the eRNA/eDNA are extracted. If filtration is carried out in the laboratory, the filters can be stored at -80°C immediately after filtration and until the eRNA/eDNA are extracted.

Note: As eRNA is very sensitive to RNAse, which is active at temperatures above -70°C, it is very important to freeze the filters in liquid nitrogen or at -80°C very quickly after filtration.

This protocole is suitable for the RNA extraction from different types of 47 mm diameter filters:

	Filter Type
	Polyethersulfone (PES)
	Polycarbonate (PC)
	Cellulose nitrate (NC)
	Cellulose mixed esters (CM)
	Cellulose acetate (CA)

- The filters should be preserved in 5 mL tubes for this RNA/DNA co-extraction protocol.

- **The following precautions must be applied:**

- Wear gloves throughout the extraction process
- - Clean the bench with a RNA/DNA-removing solution (e.g. RNase-off/DNase-off).
- Use tips with filters to avoid contaminations
- All steps have to be performed under a specific DNA/RNA-work station (sterile area equipped with air filtration and UV systems)

For any manipulation in a rare DNA/RNA room, provide complete equipment (disposable coat, cap, mask, shoe covers & gloves).

- **Pre and post extraction equipment decontamination:**

- Clean a specific DNA/RNA-work station and apply UV
- MagnetaPure 32 System (Dutscher): Visual check for residues to be removed and UV decontamination – *see instrument manual*

- **Scissors *decontamination*** *(to be done before starting the protocol, and between each filter cutting)*

- Wash them with RNase-off/DNase-off solution and rince with ethanol

- Prepare :

1. *one 50 mL tube with H_2O_2 at 10%*
2. *one 50 mL tube with molecular biology grade water*
3. *one 50 mL tube with ethanol*

- Successively dip, opening and closing the scissors into each tube.

Note: The cutting of filters is not a requirement, but it does have an impact on the extraction efficiency.

Material and rDNase preparation

1 **Material preparation**

- *To limit contamination of the kit buffers, it is recommended to aliquote them:*

- Into 50 mL tubes for MWA1, MWA2, MWA3 and MWA4
- Into 2 mL tubes for NucleoMag B-Beads solution and RNase-free H_2O

- *Tubes annotation*

- one 2 mL tube per sample for lysate collection
- Two 5 mL tubes per 8 samples for the NucleoMag B-Beads and MWA2 mix preparation
- Two 1.5 mL tubes per sample for RNA and DNA collection

- *Scissors decontamination (to be done before starting the protocole, and between each filter cutting)*

- Wash them with RNase-off/DNase-off solution and rince with ethanol
- Prepare :
1. *one 50 mL tube with H_2O_2 at 10%*
2. *one 50 mL tube with molecular biology grade water*
3. *one 50 mL tube with ethanol*

- Successively dip the scissors into each tube, opening and closing the scissors in each tube.

Note: The cutting of filters is not a requirement, but it does have an impact on the extraction efficiency.

2 **rDNase preparation** 5m

1. Reconstitution of lyophilized rDNase:
- Add 4 mL of **reaction buffer for rDNase** to the rDNase vial
- Incubate for 00:02:00 - 00:03:00 at Room temperature
- Gently shake the vials to completely dissolve the rDNase
Note: Be careful not to mix the rDNase vigorously because the rDNase is sensitive to mechanical agitation

2. rDNAse Dilution:
- Transfer the **reconstituted rDNase** to a 50 mL tube
- Add 28 mL **of reaction buffer for rDNase**
- Gently shake the tube

Note: The resulting rDNase solution will be sufficient for 96 samples. If fewer than 96 samples are to be processed, it is advisable to aliquot the rDNase as it should not be frozen and thawed more than three times. Alliquots can be store at -20 °C *for at least 6 months.*

Note: Put one aliquot in the frige a few hours before to allow slow defrosting

eRNA Plate preparation 1/2

3

30m

In this step, the buffers provided by the kit are distributed in a plate.
For RNA extraction from filtered water samples, the 12 columns of the plate are divided into 2 sections of 6 columns each, allowing up to 16 samples to be extracted per plate.

- Annotate the plate as recommended below:

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	rDNase reaction mixture	MWA3	MWA3	NucleoMag B-Beads and MWA2 mix + Lysate	MWA4	RNase-free H_2O	rDNase reaction mixture	MWA3	MWA3	NucleoMag B-Beads and MWA2 mix + Lysate	MWA4	RNase-free H_2O
Column name	D	3	3	L	4	E	D	3	3	L	4	E
	Samples between 1 to 8						Samples between 9 to 12					

Table 1: Recommended eRNA plaque annotation

Note: It is useful to mark the dividing line between columns 6 and 7 with a marker pen to provide a visual cue for filling the plate.

- Add the appropriate buffers into the appropriate wells of the plate (Table 2):

1st column / 7th column: *Will be filled during plate preparation 2/2*
2nd column / 8th column: 850 µL of **MWA3** *(1st wash)*
3rd column / 9th column: 850 µL of **MWA3** *(2nd wash)*
4th column /10th column: *Will be filled during plate preparation 2/2*
5th column / 11th column: 850 µL of **MWA4** *(3rd wash and bead drying)*

Note: The choice of elution volume is based on the expected eRNA amount. The smaller the amount, the smaller the elution volume in order to obtain more concentrated RNA.

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	*Plate preparation 2/2*	MWA3	MWA3	*Plate preparation 2/2*	MWA4	*Plate preparation 2/2*	*Plate preparation 2/2*	MWA3	MWA3	*Plate preparation 2/2*	MWA4	*Plate preparation 2/2*
Volume (µL)		850	850		850			850	850		850	
Column name		3	3		4			3	3		4	
		Samples between 1 to 8						Samples between 9 to 12				

Table 2: eRNA Plate preparation 1/2

- Film and reserve the plate at Room temperature

MWA2 preparation for RNA and DNA plate

4 - Preparation of the NucleoMag B-Beads and MWA2 mix:
- Prepare two 5 mL tube / maximum 8 samples (one for the RNA plate and one for the DNA plate)
- Add **MWA2** only, the NucleoMag B-beads will be added during plate preparation 2/2
(allow a margin of one sample or few percent for the mix preparation. For example: plan a mix for 9 samples if 8 samples are to be extracted, or add a margin of 10 %)

Sample Number	Volume NucleoMag B-Beads (µL)	Volume MWA2 (µL)
1	25	475
2	50	950
3	75	1425
4	100	1900
5	125	2375
6	150	2850
7	175	3325
8	200	3800
9	225	4275

Table 3: Volume required to prepare NucleoMag B-beads and MWA2 mix (no margin)

Sample Lysis for co-extraction

50m 5s

5 *During this step, a mechanical and chemical lysis of the sample is performed.* 50m 5s

- **For samples prefilled with buffer:**

- Collect the 5 mL tubes containing the filters

Note: If the tubes containing the filters are frozen, defrost them 00:10:00 at Room temperature

- Cut the filter into small pieces directly into the tube using decontaminated scissors

Note: Scissors must be decontaminated between each sample (see section 1 "material preparation")

- Place the tubes on the vertical vortex 00:05:00 at maximum speed
- Place the tubes into the benchtop centrifuge
- Pipette the lysate from the tube and transfer it into a 2 mL tube
- Centrifuge at 11000 rpm 00:30:00
- Replace the tubes into the rack and reserve them at Room temperature until their distribution into the plate

- **For samples without buffer:**

- Collect the 5mL tubes containing the filters from the freezer and place them On ice
- Add immediately 1100 µL of **MWA1** buffer into each tube
- Cut the filter into small pieces directly into each tube using decontaminated scissors

Note: Scissors must be decontaminated between each sample (see section 1 "material preparation")

- Place the tubes on the vertical vortex 00:00:05 at median speed
- Place the tubes on the horizontal vortex 00:05:00 at maximum speed
- Place the 5 mL tubes into the benchtop centrifuge
- Pipette the lysate from the tube (approximately 900 µL) and transfer it into a 2 mL tube
- Centrifuge at 11000 x g, 00:00:30
- Replace the tubes into the rack and reserve them at Room temperature until their distribution into the plate

eRNA Plate preparation 2/2

6 - Preparation of the NucleoMag B-Beads and MWA2 mix

NucleoMag B-Beads and MWA2 mix sediment quickly, vortex between each samples to ensure homogeneity

15m

- Vigorously vortex **NucleoMag B-Beads** tube
- For each tube containing MWA2 buffer (previously prepared), add the appropriate volume of **NucleoMag B-Beads** (*see Table 4 above*)
- Vortex

- NucleoMag B-Beads - MWA2 mix and lysate distribution

- Remove the film from the plate
- Add the appropriate solution into each well of the plate

4thcolumn /10thcolumn:

500 µL of **NucleoMag B-Beads and MWA2 mix**

450 µL of **Lysate** (supernatant from the 2 mL tubes)

6thcolumn / 12thcolumn: 50 µL or 65 µL of **RNase-free H_2O** *(RNA elution)*

- rDNase mix distribution

- Slowly agitate the **rDNase mix** tube (previously prepared- see step 2)
- Add the appropriate solution into each well of the plate

1stcolumn / 7thcolumn:

300 µL of **rDNase mix**

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	rDNase reaction mixture	*Plate preparation 2/2*	*Plate preparation 2/2*	NucleoMag B-Beads and MWA2 mix	*Plate preparation 2/2*	DNase-free H2O	rDNase reaction mixture	*Plate preparation 2/2*	*Plate preparation 2/2*	NucleoMag B-Beads and MWA2 mix	*Plate preparation 2/2*	DNase-free H2O
Volume (µL)	300			500		50 or 65	300			500		50 or 65
Solution				Lysate						Lysate		
Volume (µL)				450						450		
Column name	D			L		E	D			L		E

Samples between 1 to 8 | Samples between 9 to 12

Table 4: eRNA Plate preparation 2/2

- Film the plate and proceed immediately to the next step.

eRNA extraction with the MagnetaPure 32 System (Dutscher) 1/2

35m

7

35m

- **Extraction part 1**

- Place the plate into the MagnetaPure 32 System and insert the magnetic rod coverfor – *see instrument manual*

- **Select the appropriate program for RNA extraction part 1**

Step	Well	Name	Mix time (min)	Magnet (sec)	Wait time (min)	Volume (µL)	Mix speed	Temp (°C)
1	4	Binding	8	10	0	950	8	OFF
2	3	MWA3	2,3	5	0	850	8	OFF
3	2	MWA3	2,3	5	5	850	8	OFF
4	1	Digest DNA	2,3	0	15	300	8	37

Table 5: MagnetaPure 32 System program for NucleoMag DNA/RNA Water Kit - RNA extraction part 1

- Start the run *(The run lasts approximately* 00:35:00 *)*

eDNA Plate preparation 1/2

8 In this step, the buffers provided by the kit are distributed in a plate.
For DNA extraction from filtered water samples, the 12 columns of the plate are divided into 2 sections of 6 columns each, allowing up to 16 samples to be extracted per plate.

9 Annotate the plate as recommended below:

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	Plate preparation 2/2	MWA3	MWA3	MWA4	x	Plate preparation 2/2	Plate preparation 2/2	MWA3	MWA3	MWA4	x	Plate preparation 2/2
Column name		3	3	4	x			3	3	4	x	

Samples between 1 to 8 Samples between 9 to 12

Table 6: Recommended eDNA plaque annotation

Note: It is useful to mark the dividing line between columns 6 and 7 with a marker pen to provide a visual cue for filling the plate.

- Add the appropriate buffers into the appropriate wells of the plate.

1st column / 7th column: *Will be filled during plate preparation 2/2*
2nd column / 8th column: 850 µL of **MWA3** (1st wash)
3rd column / 9th column: 850 µL of **MWA3** (2nd wash)
4th column /10th column: 850 µL of **MWA4** (3rd wash and bead drying)
5th column / 11th column: not used
6st column / 12th column: *Will be filled during plate preparation 2/2*

Note: The choice of elution volume is based on the expected eDNA amount. The smaller the amount, the smaller the elution volume in order to obtain more concentrated DNA.

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	Plate preparation 2/2	MWA3	MWA3	MWA4	x	Plate preparation 2/2	Plate preparation 2/2	MWA3	MWA3	MWA4	x	Plate preparation 2/2
Volume (µL)		850	850	850	x			850	850	850	x	
Column name		3	3	4	x			3	3	4	x	
		Samples between 1 to 8						Samples between 9 to 12				

Table 7: eDNA Plate preparation 1/2

- Film and reserve the plate at Room temperature

eRNA extraction with the MagnetaPure 32 System (Dutscher) 2/2

30m

10 30m

- At the end of DNA digestion

- Remove the plate and add the appropriate solution into each well of the plate

1st column / 7th column: 350 µL of **MWA2**

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	MWA2						MWA2					
Volume (µL)	350						350					
Column name	D						D					
	Samples between 1 to 8						Samples between 9 to 12					

Table 9: Addition of MWA2 buffer after DNA digestion (eRNA plate)

- **Extraction part 2**

- Place the plate back into the MagnetaPure 32 System (it is not necessary to change the magnetic rod coverfor)

- **Select the appropriate program for RNA extraction part 2**

Step	Well	Name	Mix time (min)	Magnet (sec)	Wait time (min)	Volume (µL)	Mix speed	Temp (°C)
1	1	Rebind	8	10	0	650	8	OFF
2	5	MWA4	2,3	5	15	850	8	OFF
3	6	Elution	5	20	0	50 or 65	8	OFF
4	5	Release	0,5	0	0	850	10	OFF

Table 10: MagnetaPure 32 System program for NucleoMag DNA/RNA Water Kit - RNA extraction part 2

- Start the run *(The run lasts approximately* 00:30:00 *)*

eDNA Plate preparation 2/2

30s

11

30s

- Preparation of the NucleoMag B-Beads and MWA2 mix

NucleoMag B-Beads and MWA2 mix sediment quickly, vortex between each samples to ensure homogeneity

- centrifuge the lysate (prepare in step 5) at 11000 rpm during 00:00:30
- Vigorously vortex **NucleoMag B-Beads** tube
- For each tube containing MWA2 buffer (previously prepared), add the appropriate volume of **NucleoMag B-Beads** (see Table 3 above)
- Vortex

- NucleoMag B-Beads - MWA2 mix and lysate distribution

- Remove the film from the plate
- Add the appropriate solution into each well of the plate

1stcolumn / 7thcolumn:

500 µL of **NucleoMag B-Beads** and **MWA2 mix**

450 µL of **Lysate** (supernatant from the 2 mL tubes) same as eRNA lysate (step 5)

6thcolumn / 12thcolumn: 50 µL or 65 µL of **DNase-free H_2O** (DNA elution)

	1	2	3	4	5	6	7	8	9	10	11	12
Solution	NucleoMag B-Beads and MWA2 mix	*Plate preparation 1/2*	*Plate preparation 1/2*	*Plate preparation 1/2*	x	DNase-free H2O	NucleoMag B-Beads and MWA2 mix	*Plate preparation 1/2*	*Plate preparation 1/2*	*Plate preparation 1/2*	x	DNase-free H2O
Volume (µL)	500				x	50 or 65	500				x	50 or 65
Solution	Lysate				x		Lysate				x	
Volume (µL)	450				x		450				x	
Column name	L				x	E	L				x	E
	Samples between 1 to 8						Samples between 9 to 12					

Table 11: eDNA Plate preparation 2/2

- Film and reserve the plate at Room temperature

Transfer of eRNA extracts

30m

12 - At the end of the run, remove the plate and place it On ice into the DNA/RNA-workstation
- Remove the magnetic rod cover and start UV for decontamination – *see instrument manual*
- In the DNA/RNA-workstation, transfer each **eRNA extract** into a 1.5 mL tube previously annotated.

Note: It is recommended to aliquot the eRNA extracts directly to avoid freezing and thawing cycles that could degrade the eRNA.

Note: eRNA concentration and quality can be measured at this step (e.g. Nanodrop)

- Store eRNA extracts at -80 °C

eDNA Extraction step performed in the MagnetaPure 32 System

40m

13 - Place the plate into the MagnetaPure 32 System and insert the magnetic rod coverfor – see instrument manual

40m

- **Select the appropriate program to the chosen elution volume and elution temperature**

Step	Well	Name	Mix time (min)	Magnet (sec)	Wait time (min)	Volume (µL)	Mix speed	Temp (°C)
1	1	Binding	8	10	0	950	8	OFF
2	2	MWA3	2,3	5	0	850	8	OFF
3	3	MWA3	2,3	5	0	850	8	OFF
4	4	MWA4	2,3	5	15	850	8	OFF
5	6	Elution	5	20	0	50 or 65	8	56°C or OFF
6	4	Release	0.5	0	0	850	10	OFF

Table 12: MagnetaPure 32 System program for NucleoMag DNA/RNA Water Kit DNA extraction

Note: Heating to 56°C during elution gives a higher yield of DNA, but there is a risk of evaporation which reduces the volume of eluted DNA recovered

- Start the run (The run lasts approximately 00:40:00)

Transfer of eDNA extracts

14 ▪ At the end of the run, remove the plate and place it into the DNA-workstation

- Remove the magnetic rod cover and start UV for decontamination – see instrument manual
- In the DNA-workstation, transfer each e**DNA extract** into a 1.5 mL tube previously annotated

Note: DNA concentration and quality can be measured at this step (e.g. Nanodrop)

▪ Store DNA extracts at 4 °C for immediate use, or at -20 °C . or -80 °C for long-term preservation

Protocol references

NucleoMag DNA/RNA Water kit (Macherey Nagel) manual : https://www.mn-net.com/media/pdf/ce/b5/38/Instruction-NucleoMag-DNA-RNA-Water.pdf

MagnetaPure 32 Nucleic Acid Purification System (Dutscher) manual : https://pdf.dutscher.com/doc/255743/255743_MEen.pdf

eDNA extraction from water samples filtered through 47 mm diameter filters (NucleoMag DNA/RNA Water Kit - MACHEREY NAGEL) : https://www.protocols.io/view/edna-extraction-from-water-samples-filtered-throug-j8nlkox7dv5r/v1

eRNA extraction from water samples filtered through 47 mm diameter filters (NucleoMag DNA/RNA Water Kit - MACHEREY NAGEL) : https://www.protocols.io/view/erna-extraction-from-water-samples-filtered-throug-5qpvo3eq9v4o/v1