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Sterivex RNA extraction

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We use this protocol and it's working

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Abstract

A mostly automated protocol for extraction of total RNA from seawater filtered onto a Sterivex filter (Cat. No. SVGP0150). Reagents come from the Macherey-Nagel NucleoMag RNA kit (Cat. No. 744350). Automated liquid handling is performed on an eppendorf EpMotion 5075t with multi-channel pipettes. This protocol assumes that the Sterivex is sealed with tube sealant on the male end and a male-luer lock plug on the female end. Using this protocol, we routinely extract ~80 samples at a time.

The protocol has been modified to increase to an 800 µL starting volume. This normally results in less Lysis Buffer MR1, Binding Buffer MR2, and Reducing Agent TCEP supplied than is needed. Extra reagents can and should be purchased separately.

The kit manual can be found here: https://www.mn-net.com/media/pdf/23/c2/23/Instruction-NucleoMag-RNA.pdf Additional notes from the manufacturer on using the kit with the epMotion can be found here: https://www.mn-net.com/media/pdf/bd/7e/53/AN-NucleoMag-RNA-epMotion5075.pdf . Note that we have several additional modifications to their protocol.

The epMotion program is attached here.

Attachments

FILE

Application_JCVI__MN...

11KB

Guidelines

A mostly automated protocol for extraction of total RNA from seawater filtered onto a Sterivex filter (Cat. No. SVGP0150). Reagents come from the Macherey-Nagel NucleoMag RNA kit (Cat. No. 744350). Automated liquid handling is performed on an eppendorf EpMotion 5075t with multi-channel pipettes. Importantly, the epMotion must be equipped with a gripper, 1000 uL multichannel, 300 uL multichannel, an integrated themomixer, an a thermomodule. This protocol assumes that the Sterivex is sealed with tube sealant on the male end and a male-luer lock plug on the female end. Using this protocol, we routinely extract ~80 samples at a time.

The protocol has been modified to increase to an 800 µL starting volume. This normally results in less Lysis Buffer MR1, Binding Buffer MR2, and Reducing Agent TCEP supplied than is needed. Extra reagents can and should be purchased separately. Extra lysis buffer is sold in 125 mL quantities. The binding buffer is the same that is used for the DNA kit (Binding Buffer MC2) and can be purchased in 1 L quantities. Additional TCEP can also be purchased in identical vials.

Materials

MATERIALS

- RNaseZap™ RNase Decontamination Wipes **Thermo Fisher Catalog #**AM9786
- MAGNUM EX Universal Magnet Plate **Alpaqua Catalog #**A000380
- Masterblock 96 Deep Well Plate **greiner bio-one Catalog #**780286
- Flexible Tube Cutter **Catalog #**97642
- Vortex Adapter for 5mL tubes **Mobio Catalog #**13000-V1-5
- twin.tec® PCR plate 96 LoBind skirted 150 µL PCR clean **Eppendorf Catalog #** Catalog No.

Before start

- Prepare extraction sheet listing sample name, extraction number (starting from #1), and plate position (e.g. A1). We normally randomly add a few blank samples (no liquid in starting deep well plate) scattered throughout the positions.
- Clean all surfaces and tools with 70% EtOh and/or RNaseZap+MilliQ water.
- Set shaker to 33 °C

Prepare working solutions

1 **rDNase working solution:** Add 800 µL of RNase-free H_2O to the rDNase vials. Incubate for 1 min at room temperature. Gently swirl to completely dissolve. Do not mix vigourously. This can be stored at -20 °C for up to 6 months. Do not freeze / thaw more than three times.

2 **rDNase reaction mixture**: From epMotion program, quantify how much rDNase you will need for your extraction. Gently mix at the following ratio: 276 µL Reaction Buffer for rDNase: 24 µL rDNase working solution.

3 **Reducing Agent TCEP:** Add 750 µL of RNase-free H_2O to the TCEP vial and incubate for several minutes at room temperature. Mix the vial to dissolve the TCEP completely. Store dissolved TCEP at -20 °C.

4 **Lysis Buffer** (N = number of samples plus extra for pipetting; X = quantity of internal standard mix (usually 1-2.5 µL):

In sterile container, add 800 µL x N of Lysis Buffer MR1. Add 14 µL x N TCEP. Add X x N µL of internal standard mix. Mix well

Lysis

5 Keep sterivex on dry ice. In each, remove luer plug and pipette the per sample volume prepared in step 1 into each. For example, if 800 µL MR1 + 14 µL TCEP + 1 µL internal standards were used, add 815 µL. Dispense quickly as the lysis buffer will freeze upon contact. Replug with luer lock. Place luer-side down into 1.5 mL tube rack.

6 Incubate at 33 °C and shake gently for 00:10:00 .

7 Vortex for 00:05:00 using adapter for 5 mL tubes.

8 Transfer lysate to sterile 1.5 mL tubes labeled with extraction numbers.

8.1 Label each sterviex with its extraction number.

8.2 Pop the lid with the male end off the end of the sterivex using the tube cutters. The tube cutters will fit in the lip between the lid and the rest of the unit. Apply pressure at an upward angle and it should release. Continue to cut around the top if needed.

8.3 Rub the filter on the inside of the sterviex to release as much of the lysis buffer and material as possible. Then pipette as much liquid as possible from inside the sterviex into the corresponding 1.5 mL tube.

9 Clear lysate by centrifugaion at 5600 x g, 00:06:00 .

Note

This is a good time to prepare your buffers. The epMotion will calculate your buffer volumes depending on the number of samples. Add a few mL more than is requested and enter that as the volume supplied. We premix the beads and Binding Buffer MR2 at a ratio of 28 µL beads:772 µL MR2. Note that this keeps the bead amount the same as specified in the manual while increasing the amount of MR2.

10 Transfer to another set of numbered 1.5 mL tubes. Remove as much supernatent as possible without disturbing the pellet.

11 Align 1.5 mL tubes in rack in plate position. Set adjustable spacer multichannel pipette to 800 µL and transfer **no more** than 800 µL into the deep well plate.

12 Transfer deep well plate to the epMotion and run program. The final elution is always into a twin.tec LoBind PCR plate (see materials).

Note

Reservoir rack layout (left to right):
1. MR2 with beads
2. MR3
3. MR4
4. MR4
5. MR5
6. MR2 without beads
7. DNase

Note

Step 4 in the program stops it. At this point with a P1000 multichannel pipette, throughly pipette up and down to mix the bead solution then continue the run.

13 **Quality:** Analyze 1 µL on Agilent Tapestation with RNA screentape.

14 **Quantification:** Make a 1:10 dilution plate (20 µL total volume) using the elution buffer MR5 (RNase-free H_2O). Quantify the dilution plate with RiboGreen assay and use that to calculate the concentration and total RNA in the original plate.