RNA Extraction from Sterivex filters

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Abstract

RNA extraction from water collected on Sterivex. The DNeasy PowerWater Sterivex kit is used and modified specifically for RNA. Also includes modifications for samples preserved in RNAlater.

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Guidelines

What you will need:

-Kit: Qiagen DNeasy PowerWater Sterivex kit (formerly MoBio PowerWater DNA Isolation for Sterivex (Catalog# 14600-50-NF))

-Kit: Invitrogen Turbo DNase (Catalog# AM1907)

-B-mercaptoethanol (BME) *

-65ºC incubator

-70ºC incubator

-4ºC refrigerator

-Vortex

-100% RNase free EtOH

-RNase free H_2O

-60 mL or 3mL sterile syringes (If using RNAlater solution)

*** See warnings**

Before start

This protocol is modified from a MoBio Protocol. You will need:

a.) MoBio PowerWater DNA Isolation for Sterivex Kit (Catalog# 14600-50-NF)

b.) Invitrogen Turbo DNase (Catalog # AM1907)

Protocol

Preparation

Step 1.

Add solution ST1A to bottle ST1B (provided in kit).

NOTES

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Only required the first time you use ST1B, it can be kept at 4°C for storage when not in use.

Preparation

Step 2.

Warm solutions ST2 and ST4 prior to use at 65°C for 5-10 minutes. Use while warm.

NOTES

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Solutions ST2 and ST4 are salty solutions and precipitates, must be dissolved prior to use.

Preparation

Step 3.

If Sterivex were capped with clay at the outlet and no screw cap is available, use electrical tape to secure the opening. The clay may not hold during the entire extraction process and this will prevent your lysate from leaking.

Preparation

Step 4.

(900µL) ST1B/Beta-mercaptoethanol (BME) solution will be needed per sample and every sample. Prepare fresh daily as needed.

Combine (20µL) of BME with (880µL) ST1B.

AMOUNT
900 µl Additional info:

For samples preserved in RNAlater

Step 5.

Remove RNAlater from Sterivex. If clay was used to plug the outlet, unplug with a sterile needle.

For samples preserved in RNAlater

Step 6.

Connect a sterile syringe to the inlet and flush Sterivex with 50mL RNase free H_2O.

* Make sure all water is pushed out before moving forward.

NOTES
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Be sure to Re-plug or Re-cap the outlet.

Alternative for RNAlater removal if pushing liquid through the Sterivex is not an option

Step 7.

Pull back the plunger of a 3mL syringe to fill the barrel with 1mL (or more) air.

Attach the syringe to the inlet end of the Sterivex and hold the unit vertically with the syringe at the bottom to allow as much of the lysate as possible to be near the inlet.

Step 8.

Push air into the unit until there is a resistance and release the plunger. You may not feel resistance but if you push and pull air in and out a couple of times, it should work to pull the lysate into the syringe.

DO NOT FORCE AIR THROUGH THE STERIVEX.

Step 9.

Collect the RNAlater in a centrifuge tube if cells detach from the filter.

Centrifuge to pellet cells and discard the supernatant.

Step 10.

Resuspend cells in the 900µL ST1B/BME solution, and add back into the Sterivex through the inlet. Insert pipette completely into the inlet so that the pipette tip is visible inside the unit just above the membrane.

NOTES
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ST1B/BME solution listed above.

If RNAlater was not used.

Step 11.

Remove inlet cap and add (900µL) of the ST1B/BME solution use a pipette tip. Insert pipette completely into the inlet so that the pipette tip is visible inside the unit just above the membrane.

AMOUNT
900 µl Additional info:

Continue as follows regardless of RNAlater utilization.

Step 12.

Recap the inlet and secure the Sterivex filter unit horizontally with the inlet facing out.

Step 13.

Vortex at minimum speed for 5 minutes.

DURATION
00:05:00

Step 14.

While still attached to the vortex adapter, rotate the Sterivex 180 degrees from the original position.

NOTES
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Marking initial position on the inlet cap may be useful.

Step 15.

Vortex at minimum speed for an additional 5 minutes.

DURATION
00:05:00

NOTES
Ashley Humphrey 06 Dec 2016

An increase in time may be necessary if many cells are still attached to the filter.

Step 16.

Set the Sterivex with the inlet facing up and remove the inlet cap.

Step 17.

Add (900µL) of ST2 solution using a pipette tip and recap the inlet.

AMOUNT
900 µl Additional info:

NOTES
Ashley Humphrey 06 Dec 2016

Recall ST2 solution must be warmed.

Step 18.

Incubate the Sterivex at 70ºC for 10 minutes, be sure to place unit for equal distribution of heat.

DURATION
00:01:00

Step 19.

Cool the unit at room temperature for two minutes. Ensure caps are on tightly, as they may loosen after heating.

NOTES
Ashley Humphrey 06 Dec 2016

It is importatnt to cool the unit before re-tightening the caps to minimize warping of the inlet.

Step 20.

Secure the Sterivex on the vortex with the inlet facing out n. Vortex at maximum speed for 7.5-10 minutes.

NOTES
Ashley Humphrey 06 Dec 2016

Critical step for removing the remaining cells from filter and effectively lysing the cells.

Step 21.

Using the provided 3mL syringe, pull back the plunger with 1mL (or more) of air. Attach the syringe to the inlet of the Sterivex and hold the unit vertically with the syringe at the bottom to allow as much of the lysate as possible to be near the inlet.

Step 22.

Push air into the unit until there is a resistance and release the plunger. Repeat air resistance process until you are able to pull the lysate out into the syringe. *DO NOT FORCE AIR THROUGH THE

STERIVEX*

Step 23.

Add the lysate to the 5mL PowerWater Sterivex glass Bead Tube.

Step 24.

Secure the BeadTube with the lysate to the vortex.

Step 25.

Vortex at maximum speed for 7.5-10 minutes.

NOTES
Ashley Humphrey 06 Dec 2016

This step is critical for homogenization and lysis of the cells.

Step 26.

Centrifuge the tube at 3600 x g for 2 minutes at room temperature.

DURATION
00:02:00

Step 27.

Placing the pipette tip down into the beads against the bottom of the tube, transfer all the supernatant out to a clean 2.2mL collection tube provided in kit. Pipet more than once to ensure removal of supernatant.

Any carry over of beads will not affect the subsequent steps.

NOTES
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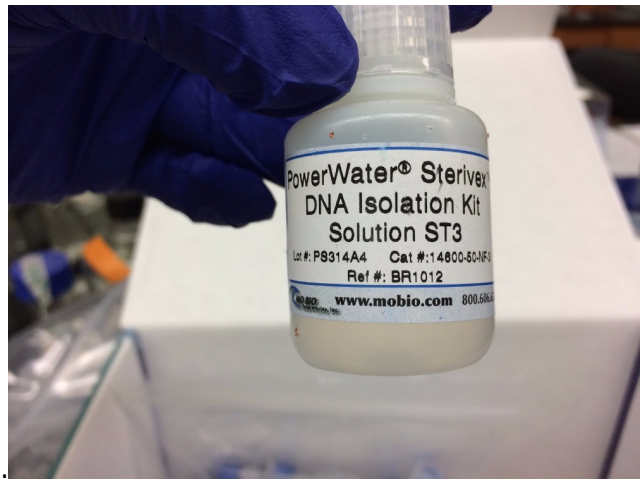
Expect to recover about 1.5mL of supernatant.

Step 28.

Add 300µL of ST3 solution and vortex briefly to mix



AMOUNT
300 µl Additional info:

Step 29.

Incubate at 4ºC for 5 minutes.

DURATION
00:05:00

Step 30.

Centrifuge the tubes at 13000 x g for 1 minute.

DURATION
00:01:00

Step 31.

Avoiding the pellet, transfer the supernatant to a clean 5mL collection tube.

Step 32.

Add 1.5mL of 100% RNase-free EtOH and 1.5mL of warmed ST4 Solution, then mix.

Step 33.

Place a binding column (provided) into a 2.2mL microcentrifuge tube (provided). Add previous solution (step 32) to the binding column (800µL) at a time. Centrifuge at 13000 x g for 1 minute and discard the supernatant each time to collect the RNA in the filter.

NOTES
Ashley Humphrey 19 Jan 2017

This step replaces the need to use the vacuum manifold. The vacuum manifold tends to get clogged up when the samples are salty (i.e. with use of RNAlater) and in general takes a long time to actually filter.

Step 34.

When all of the solution from step 32 has been filtered through the binding column completely, centrifuge one final time for 1 minute to ensure the filter is completely dry.

DURATION
00:01:00
Step 35.

Transfer the binding column to a new 2.2mL microcentrifuge tube.

Step 36.

Add 800 µL of Solution ST5 to the binding column, and let sit for 1 minute.

AMOUNT
800 µl Additional info:
Step 37.

Centrifuge for 1 minute at 13000 x g.

DURATION
00:01:00
Step 38.

Discard the supernatant and centrifuge again for 1 minute to ensure filter is completely dry. Discard residual supernatant.

DURATION
00:01:00
Step 39.

Shake solution ST6 to mix well. Add 800 µL of ST6 and let sit for 1 minute.

AMOUNT
800 µl Additional info:
DURATION
00:01:00

Step 40.

Centrifuge ST6 solution for 1 minute at 13000 x g.

DURATION
00:01:00
Step 41.

Discard the supernatant, and centrifuge again for 1 minute to ensure filter is completely dry. Discard residual supernatant.

DURATION
00:01:00
Step 42.

Again, add 800 µL of Solution ST5 to binding column. Let sit for 1 minute.

AMOUNT
1 ml Additional info:
DURATION
00:01:00
Step 43.

Centrifuge solution ST5 for 1 minute at 13000 x g.

Step 44.

Discard the supernatant and centrifuge again for 1 minute to ensure the filter is completely dry. Discard the residual supernatant.

If RNAlater was used.
Step 45.

Add 800µL of 100% RNase free EtOH to the binding column. Let sit for 1 minute.

If RNAlater was used.
Step 46.

Centrifuge 100% EtOH for 1 minute at 13000 x g.

If RNAlater was used.
Step 47.

Discard the supernatant.

Continue as follows regardless of RNAlater utilization.
Step 48.

Centrifuge again for 2 minutes to ensure filter is completely dry. Discard residual supernatant.

Step 49.

Place binding column into a new 1.5 mL centrifuge tube.

Step 50.

Add 100 µL of RNase-free water to the center of the filter. Let sit for 1-5 minutes.

AMOUNT
100 µl Additional info:

Step 51.

Centrifuge at room temperature for 1 minute at 13,000 x g.

DURATION
00:01:00

Step 52.

RNA is collected this time, discard the binding column.

Step 53.

Use Turbo DNase kit for DNase treatment. Follow manufacturer's protocol.

LINK:
http:/http://tools.thermofisher.com/content/sfs/manuals/cms_055740.pdf

Step 54.

RNA ready to use, or store at -80º C.

Warnings

-WORK IN FUME HOOD FOR ALL STEPS INVOLVING **B-Mercaptoethanol (BME)**

https://www.sciencelab.com/msds.php?msdsId=9924612

-Wear gloves and avoid skin contact with all reagents in MoBio and Invitrogen kits.