RNA Isolation from Viral-infected Chlorella NC64A

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Abstract

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Guidelines

Materials:

1.) NC64A mid-log phase cells, 1-2 X 10^7 cells/ml

2.) PBCV-1 or other virus

3.) liquid nitrogen

4.) spatulas, baked

5.) 2 ml microfuge tubes, sterile, RNAse-free (use gloves w/unopened bag, autoclave)

6.) 0.25-0.30 mm glass beads, 250 mg each sample, baked

7.) chloroform (w/o isoamyl alcohol)

8.) isopropanol (use a bottle exclusively for RNA work)

9.) 75 % EtOH (use EtOH set aside for RNA work only, dilute w/ DEPC-treated water)

10.) 10X MOPS buffer = 400 mM MOPS, 100 mM NaOAc, pH 7.0 (DEPC-treated)

10X MOPS:

92.48 g MOPS

13.61 g NaOAc×$3H_20$

Adjust to pH 7.0 w/ NaOH

dd-H_20 to 1 L

11.) 1 M $MgCl_2$ solution (DEPC-treated)

12.) DNAse I (RNAse-free)

13.) dd-H_20 (DEPC-treated)

14.) 1:1 phenol/chloroform (prepared w/o additives for RNA work exclusively)

Note: DEPC-treat solutions by addition of 0.1% diethylpyrocarbonate, dissolving DEPC by vigorous mixing, incubating 1 h at 37°C or overnight at 25°C, and autoclaving. Caution: DEPC is toxic.

The RNA isolation procedure shown is a modification of the TRIZOL procedure, which is based on a procedure originally developed by Chomczynski and Sachi [Anal. Biochem. **162**, 156-159 (1987)].

Protocol

Step 1.
Grow NC64A to mid-log phase.

Step 2.
Concentrate to 1 x 10^8 cells/ml.

Step 3.
Use 30 ml concentrated cells per time point (3 x 10^9 cells91 mg cells wet weight).

Step 4.
Infect with virus at m.o.i.=5 for appropriate time.

Step 5.
Harvest infected cells by centrifugation at 5000 rpm, 5 min., 4°C.

DURATION
00:05:00

Step 6.
Discard supernatant and flash freeze pellet in liquid nitrogen.

Step 7.
Store at -80°C until ready to proceed to step #8.

Step 8.
Take 1 to 2 samples from the freezer at a time (keep on ice), break pellets loose with spatula, and add 1 ml TRIZOL reagent to each tube. Work as quickly as possible to prevent RNA degradation.

Step 9.
Break pellets up with spatula and vortexing.

Step 10.
Pipet into 2 ml microcentrifuge tube(s) containing 250 mg of 0.25-0.30 mm baked glass beads.

Step 11.
Immediately vortex at highest speed 5 min., 4°C.

DURATION
00:05:00

Step 12.

Place at -80°C overnight.

Step 13.
Repeat steps #8-12 for the remaining samples.

NOTES
Irina Agarkova 24 Mar 2016

Note: samples may be stored at -80°C for up to 1 month at this point.

Step 14.
Thaw samples and vortex another 5 minutes.

DURATION
00:05:00

Step 15.
Add 250 µl chloroform to samples, then vortex briefly.

Step 16.
Let stand at room temp. 3 min.

DURATION
00:03:00

Step 17.
Centrifuge samples at 12,000 x g, 15 min., 4°C.

DURATION
00:15:00

Step 18.
Withdraw the RNA-containing aqueous upper phase and deliver to fresh microcentrifuge tube.

Step 19.
Add 500 µl isopropanol.

Step 20.
Let stand 10 min. at room temp.

DURATION
00:10:00

Step 21.
Centrifuge at 12,000 x g, 10 min., 4°C.

DURATION
00:10:00

Step 22.
Discard supernatant.

Step 23.
Wash pellet with 75% EtOH with vortexing.

NOTES
Irina Agarkova 14 Apr 2016

Note: samples may be stored for one year at -20°C in the 75% EtOH wash at this point.

Step 24.
Centrifuge 12,000 x g, 5 min., 4°C.

DURATION
00:05:00

Step 25.
Dry pellets in vacuum dessicator 5-10 min. DO NOT DRY COMPLETELY.

DURATION
00:10:00

Step 26.
DNAse each sample by resuspending each pellet in 250 µl dd-H20.

NOTES
Irina Agarkova 24 Mar 2016
Note: Adding part of the dd-H_20 alone first to pellet. This seems to make the pellet go into solution faster.

Step 27.
Add 250 µl of 2X MOPS, 20mM $MgCl_2$ (2X), and 100-200 U RNAse-free DNAse I.

Step 28.
Incubate 1-2 h on ice.

DURATION
02:00:00

Step 29.
Extract with equal volume of phenol:chloroform (500 µl).

Step 30.
Centrifuge at 12,000 x g, 5 min., 4°C.

DURATION
00:05:00

Step 31.
Withdraw aqueous phase, add NaOAc to 0.3 M.

Step 32.
Add 1 ml isopropanol.

Step 33.
Let stand 10 min., room temp.

DURATION
00:10:00

Step 34.

Centrifuge up to 12,000 x g, 10 min., 4°C.

DURATION
00:10:00

Step 35.
Discard supernatant.

Step 36.
Wash pellet with 75 % EtOH with vortexing.

Step 37.
Centrifuge 12,000 x g, 5 min., 4°C.

DURATION
00:05:00

Step 38.

Dry pellets in vacuum dessicator 5-10 min. Do not dry completely.

DURATION

00:10:00

Step 39.

Resuspend pellets in 40-50 µl water.

NOTES

Irina Agarkova 24 Mar 2016

Freezing at -80°C and then thawing seems to help the RNA go into solution better.

Irina Agarkova 31 May 2016

Note: expect maybe 2-5 µg/µl yield.

Warnings

Caution: DEPC is toxic.