394 SW 12th Avenue
Deerfield Beach, FL 33442
www.oceanridgebio.com
array@oceanridgebio.com
Phone: 754-600-5128
Atl. Phone: 754-600-5139

DNase Digestion and Clean-Up for Gene Expression Profiling

Warning: Not for MicroRNA

Note, adjust Dnase digestion volume to 50 ul if you have less than 15 ug of RNA.

1. Dilute 30 ug of RNA in a volume of 84 ul of Qiagen RNase-free water.
2. Add 10ul of Invitrogen 10X Dnase Digestion Buffer.
3. Add 2ul of Rnase Block (Rnase Inhibitor) -mix by gentle vortexing.
4. Add 4ul of Ambion Rnase-free Dnase I.
5. Mix by inversion or flicking and spin briefly (do not vortex).
6. Incubate at 37C for 30 min.
7. Add 4.0ul of 0.5 M EDTA pH 8, vortex and spin down.
8. Adjust volume of Dnased-RNA to 100 ul with Qiagen water if necessary.
9. Combine DNased digest RNA with 350 ul of buffer RLT and mix by gentle vortexing (don't centrifuge).
10. Combine RNA/RLT mixture with 250 ul of 100% ethanol.
11. Mix by gentle vortexing (don't centrifuge).
12. Apply the sample to an Rneasy MinElute spin column in a 2ml collection tube.
13. Close the tube gently and centrifuge for 1 min at >10,000 g.
14. Transfer the spin column into a new 2ml collection tube.
15. Pipet 500 ul buffer RPE onto the spin column.
16. (Apply around rim of column to make sure the area is washed)
17. Close the tube gently, and centrifuge for 1 min at >10,000 g to wash the column.
18. Discard the flow-through and reuse collection tube.
19. Pipette 500 ul of 80% ethanol to the Rneasy minelute spin column.
20. Close the tube gently, and centrifuge for 1 min at >10,000 g.
21. Discard flow-through and collection tube.
22. Transfer the Rneasy minelute spin column into a new 2 ml collection tube.
23. Close the cap of the spin column, and centrifuge in a microcentrifuge at 16,000 g or max speed for 3 min.
24. Discard flow-through and collection tube.
25. Repeat steps 21-23 one more times (important to remove any trace of ethanol).
26. To elute, transfer the spin column to a new 2 ml capless collection tube.
27. Pipette 14 ul Rnase-free water directly onto the center of the silica-gel membrane.
28. Close the tube gently, incubate 1 minute on bench and centrifuge for 1 min at max speed to elute
29. Measure OD of 1:50 dilution of RNA at 260 nm and 280 nm.
30. Freeze at -70C or ship to ORB on dry ice.

Shipping

Select a box for shipment that has an external dimension of at least 25 cm in each dimension. The box should have a sturdy cardboard exterior and inner Styrofoam box with wall thickness of at least 2.5 cm. Fill the storage box with 1.5 kg of dry ice. Cool a 133 mm x 133 mm x 48 mm vial storage box in the shipping container. Transfer the frozen sample tubes to the vial storage box. Fill in the free areas of the vial storage box with dry ice and secure the vial storage box top over the samples using wire or string. Fill the Styrofoam shipping container with additional dry ice, and packing peanuts (if necessary) in order to minimize free space in the package. The total dry ice content should be at least 2.5 kg. Use FedEx overnight delivery to ship the package to ORB at the address below. Affix the dry ice sticker to the exterior of the package and record the same dry ice weight on the sticker as was used during set-up of the shipment (may require metric to English unit conversion).

Attention: David Willoughby

Ocean Ridge Biosciences, LLC
394 SW 12th Avenue
Deerfield Beach, FL 33442
Phone: 754-600-5128
Emergency Phone: 561-427-5548