

Double Stranded RNA Purification

1. Weigh out 100 mg CF11 cellulose per g fresh weight of tissue to be used, and place in a BioRad Econocolumn (catalog number 731-1550). An ideal amount of tissue to use is 5 g fresh weight. This will require enough CF11 cellulose to fill the lower portion of the spin column.
2. Grind tissue in liquid nitrogen until completely pulverized. Transfer powdered tissue to a tube containing 2 ml of extraction buffer and 2 ml of phenol:chloroform per original g tissue (for 5 g of tissue, use 10 ml of buffer and 10 ml of phenol:chloroform. For dry tissue you may need to increase this volume .
- 3 Mix for about 10 minutes at RT. Centrifuge for 10 minutes in the table-top, at top speed.
4. Remove aqueous phase and repeat phenol extraction.
5. Remove final aqueous phase into a 12 ml Falcon tube, measure carefully, and add room temperature absolute ethanol to 16.5% (multiply volume by 0.198). If only 95% ethanol is available multiply by 0.21).
6. Add to the CF11 powder, close the column, and mix thoroughly. Remove top and bottom closures, place column in Falcon tube, and spin in a tabletop centrifuge at 2,000 rpm for 30 seconds (just long enough to spin through all of the liquid).
7. Seal bottom of column and fill with application buffer. Close top, and mix thoroughly. Be sure to secure both closures of the column, as the EtOH will build some pressure in the column during mixing. Spin as in step 6. Repeat wash a total of 3 times.
8. Add 4.5 ml of elution buffer and mix, as in step 7. Place column over a clean 15 ml Corex tube, and spin for 1 minute.
9. Add 0.5 ml of 3M NaOAC and 10 ml of EtOH, and precipitate overnight at -20°C.
10. Spin down ds RNA and resuspend in appropriate volume of 0.1 mM EDTA. If the first precipitation is in a Corex tube it is a good idea to reprecipitate it in a microfuge tube.
11. DNase digestion (optional) can be done to remove any trace amounts of residual DNA if this is important for the further use of the dsRNA.
12. Check the dsRNA on a 1.5% agarose gel or a 5% polyacrylamide gel in 1 X TBE or 1 X TAE. The TBE gel can be run at 250 volts, and will take about 15 minutes to run a mini Protean II gel. The ds sat RNA (~350 nt) will run approximately with the BPB dye. Even large dsRNA, like potyvirus dsRNA will enter the gel, whereas the residual DNA

will remain in the well. (You cannot always distinguish DNA and large dsRNA on an agarose gel).

Buffers:

Extraction buffer:

0.1 M NaCl
50 mM Tris, pH 8
1 mM EDTA, pH 8
1 % SDS
0.1 % 2-Mercaptoethanol
(optional) add ~1% dry powder PVPP

Application buffer:

0.1 M NaCl
50 mM Tris, pH 8
0.5 mM EDTA, pH 8
16.5 % Ethanol

Elution buffer:

0.1 M NaCl
50 mM Tris, pH 8
0.5 mM EDTA, pH 8