RNA Isolation by the Guanidine Thiocyanate/CsCl Method
We highly recommend this protocol for prostate RNA

Solution Preparation

1.0 M Sodium citrate pH 7
Sodium citrate 29.4g
DEPC water to 95ml

Adjust the pH to 7 and bring the volume to 100mls. Filter through .2 μm filter and autoclave.

0.5M EDTA pH 8.0
Sodium EDTA 18.6g
DEPC water to 90ml

To facilitate dissolution of the EDTA, keep the pH near 7 with NaOH. Once all the EDTA has gone into solution adjust the pH to 8.0. Filter.

5.0 M NaCl
NaCl 29.2g
water to 100ml
Treat with 100μl DEPC and autoclave.

Guanidine thiocyanate solution				[Final]
Guanidine thiocyanate	30g	60g	180g	5.1M
10% n-lauroylsarcosine*	2.5ml	5ml	15ml	0.5%
1.0M sodium citrate	2.5ml	5ml	15ml	50mM
0.5M EDTA pH 8	5.0ml	10ml	30ml	50mM
30% Antifoam A	.17ml	.333ml	1.0ml	0.1%
Sterile water to	50ml	100ml	300ml	

* added after tissue homogenization to decrease foaming, 50μl per 1 ml

Allow compounds to dissolve. Heating to 37C will facilitate this process.
Filter using 0.4μm filter, followed by .2μm.
Just before use add ß-mercaptoethanol 5μl per ml of final homogenate.

Cesium chloride solution			[Final]
Cesium chloride	48g	96g	5.7M
Sodium EDTA	1.68g*	3.36g**	0.1M
Sterile water to	50ml	100ml	

*or 10 (**20) mls of 0.5M EDTA pH 8 respectively
This solution should be filtered as for the Guanidine. Treat with DEPC and autoclave.

Homogenization

Currently, the most consistently successful method for prostate RNA isolatio
is to remove the GU tract en bloc while under anesthesia, perform the dissection on a frozen culture dish, dip the tissue in guanidine solution prior to placing it on the foil to freeze and then homogenize immediately.

1. Homogenize fresh tissue in 3mls of guanidine solution (remember to add ß-mercaptoethanol before and sarcosyl afterwards) on speed 4 for a 5-10 seconds

2. Place 2ml of CsCl solution in Beckman centrifuge tubes (13x51mm).

3. Aspirate homogenate with a RNase free transfer pipette and carefully laye over the CsCl.

4. Bring liquid level up to 1/4" below the top with guanidine solution, plac in SW50.1 tubes, and balance with caps to within .01-.02g.

5. Centrifuge @ 36,000 RPM, Temp 15-35, for 18-20 hours. Allow to come to a stop without the brake.

6. Aspirate liquid off until the last 300-400µl and then invert tube to drai the remainder. Cut the bottom of the tube off with a razor blade and resuspend pellet in 270µl of DEPC water.

7. Add 30µl of RQ1DNase buffer and 2 µl of enzyme (2 units), incubate at 37 for 30 minutes.

8. Phenol-Chloroform extract with 300µl (3:1) and vortex vigorously for 30-60sec.

9. Spin for 5min @ 4C. Remove 80% of the aqueous phase (300µl).

10. Add 1/2 volume of 7.5M Ammonium acetate (150µl) and 900µl 100% EtOH.

11. Spin for 30min at 4C, wash twice with 80% EtOH with 5 min spin between each wash.

12. Remove all EtOH, air dry for 10 minutes, and resuspend in 30-50µL DEP water to read OD.