Extraction of mf RNA from samples contaminated with host blood and/or cellular material:

Collection: Samples should be frozen in 1.5ml tubes in a volume of less than or equal to 100ul. If the total volume is over 100ul Trizol LS should be used instead of RNAzol.

Part 1: RNAzol extract, modification of

Chomczynski P, Sacchi N. Single-step method of RNA isolation by guanidinium thiocyanate-phenol-chloroform extraction. AnalBiochem 1987;162:156–9.

RNAzol solution : For 100ml

Dissolve 47.264g Guanidine isothiocyanate in 40ml 25mM Sodium Citrate pH 7.0 (DEPC treated).
Add 5ml 10% Sarkosyl solution (N Lauroylsarcosine)
Bring to 100ml with 25mM Sodium citrate
Filter sterilize (.22um nylon)
Cover with aluminum foil and store at 4°C

Best if used fresh but can be stored for up to 3 months (DNA contamination will increase with the age of the RNAzol solution)

Extraction solution: 1ml RNAzol solution
1ml acid phenol
100ul 2M NaOAc pH 4.0 (DEPC treated)
15ul β-ME
Keep on ice

Remove samples from the freezer and keep on dry ice.

1. Add 750ul extraction solution and grind up frozen pellet with a pestle (Kontes®) until no tissue is visible. Optional- homogenize with a rotor-stator homogenizer.
2. Add 200ul $CHCl_3$ and invert 20 times
3. Ice 20min., invert 20 times
4. Centrifuge 20min. at 4°C and max speed and transfer aqueous phase to a new tube
5. Add an equal vol of isopropanol, mix by inversion, quick-spin, and precipitate at least 1hr at -20°C (preferably O/N)
6. Do not invert or mix tube. Centrifuge 30min. at 4°C and max speed, pour off supernatant
7. Wash with 800ul -20°C 80% EtOH (in DEPC water), centrifuge 5min. at 4°C and max speed, pour off supernatant
8. Repeat step 7
9. Remove as much residual EtOH as possible without disturbing the pellet and air dry at 37°C for 15min.
10. Resuspend on ice in 100ul nuclease free water, do not heat. Store at -80°C or proceed to part 2.

Part 2: Zymo Research RNA Clean and Concentrator Column (Zymo Research Corp, www.zymoresearch.com). The columns come in different binding capacities so be sure to get the appropriate size.

Note: It is possible to go directly from the aqueous phase of the above organic extraction right into the column, however, volume issues are avoided and yield is more consistent if the RNAzol extraction/precipitation is completed as above.

Perform the General or Small RNA Elimination protocol following the manufacturer's instructions, including the in-column DNase step.

The DNase incubation can be done at room temp.

A second elution is recommended to increase yield.

The total elution volume used should be adjusted for the expected amount of RNA.