**Isolating RNA**

1. Add an equal volume of 70% ethanol to the filtrate and vortex the tube for 5 seconds until the filtrate and ethanol are mixed thoroughly.
2. Transfer up to 700 µl of this mixture to an RNA Binding Spin Cup that is seated in a fresh 2-ml receptacle tube and cap the spin cup. Vigorously flex the hinge of the spin cup, prior to closing it, so that it becomes flexible and the cap can be firmly seated in the tube, to reduce the chance of leakage during vortexing.
3. Spin the mixture in a microcentrifuge at maximum speed for 30-60 seconds.
4. Remove and retain the spin cup and discard the filtrate. Replace the spin cup in the receptacle tube. For samples homogenized in >350 µl of Lysis Buffer, repeat steps 2-4 with the remaining mixture.

**Note:** *The RNA was protected in previous steps from RNases by the presence of guanidine thiocynate.*

1. ***DNase Treatment*** This procedure is recommended for RT-PCR applications.
   1. Add 600 µl of 1 x Low-Salt Wash Buffer and snap the cap of the receptacle tube onto the spin cup. Spin the sample in a microcentrifuge at maximum speed for 30-60 seconds.
   2. Remove and **retain the spin cup** and discard the filtrate. Replace the spin cup in the receptacle tube and cap the spin cup. Spin the tube in a microcentrifuge at maximum speed for 2 minutes.
   3. Prepare the DNase solution by gently mixing 50 µl of DNase Digestion Buffer with 5 µl of reconstituted RNase-Free DNase I.

**Note**: *Gentle mixing is necessary because the DNase I is very sensitive to denaturation.*

* 1. Add the DNase solution directly onto the matrix inside the spin cup and cap the spin cup.
  2. Incubate the sample at 30°C for 15 minutes in an air incubator.

1. Add 600 µl of 1x High-Salt Wash Buffer to the spin cup and cap the spin cup. Spin the tube in a microcentrifuge at maximum speed for 30-60 seconds.

**Caution:** *The High-Salt Wash Buffer contains irritant guanidine thiocynate.*

1. Remove and **retain the spin cup** and discard the filtrate. Replace the spin cup in the receptacle tube.
2. Add 600 µl of 1 x Low-Salt Wash Buffer and cap the spin cup. Spin the tube in a microcentrifuge at maximum speed for 30-60 seconds.
3. Remove and **retain the spin cup** and discard the filtrate. Replace the spin cup in the receptacle tube.
4. Add 300 µl of 1 x Low-Salt Wash Buffer and cap the spin cup. Spin the tube in a microcentrifuge at maximum speed for 2 minutes to dry the matrix.
5. Transfer the spin cup to a 1.5-ml microcentrifuge tube and discard the 2-ml receptacle tube.
6. Add 30-100 µl of Elution Buffer directly onto the center of the matrix inside the spin cup and cap the spin cup. Incubate the tube for 2 minutes at room temperature. Spin the tube in a microcentrifuge at maximum speed for 1 minute. Repeat this elution step to maximize the yield of RNA.

**Note:** *The Elution Buffer must be added directly onto the matrix of the spin cup to ensure that the Elution Buffer permeates the entire matrix.*

*The RNA yield can be increased by using Elution Buffer warmed to 60°C.*

The purified RNA is in the Elution Buffer in the microcentrifuge tube. The RNA can be stored at -20°C for up to one month or at -80°C for long-term storage.