OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® FFPE RNA Kit

M2555-00	5 preps
M2555-01	50 preps

Mag-Bind® FFPE RNA 96 Kit

M2551-00	1 x 96 preps
M2551-01	4 x 96 preps
M2551-02	20 x 96 preps

August 2012

For research use only.Not intended for diagnostic testing.

Mag-Bind® FFPE RNA
Mag-Bind® FFPE RNA 96

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Manual Revision: May 2013

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Introduction

The Mag-Bind® FFPE RNA kit and the Mag-Bind® FFPE RNA 96 kit provide a rapid and easy method for the isolation of total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. Due to fixation and embedding procedures, nucleic acids in FFPE samples are heavily fragmented and modified by formaldehyde. While the Mag-Bind® FFPE RNA kit and the Mag-Bind® FFPE RNA 96 kit are optimized to minimize the effect of the formaldehyde modification, it is not recommended to use the RNA purified with this kit for downstream applications that requires full length RNA.

Principle

The Mag-Bind® FFPE RNA kit and the Mag-Bind® FFPE RNA 96 kit combine high efficient binding properties of Mag-Bind® technology with a specially designed buffer system to isolate total RNA sample from FFPE sample. There are two protocols included in this manual. The standard protocol uses a heating step instead of xylene to remove paraffin from the sample. The alternative protocol uses traditional xylene extraction to remove paraffin.

Samples are first lysed in RML Buffer with digestion of Proteinase K. The lysate is then mixed with MFB Buffer and magnetic particles to bind the nucleic acid on the surface of the Mag-Bind® Particles SC. Genomic DNA is removed by DNase I digestion. After two wash steps, purified RNA is eluted with RNase-free water.

Starting Materials

Since standard formalin fixation and paraffin embedding procedures cause significant fragmentation of nucleic acids. We recommend following guidelines to limit the extent of DNA/RNA fragmentation: 1) Use 4-10% formalin to fixate tissue samples; 2) Limit the fixation time to 14-24 hours; 3) Completely dehydrate samples before embedding. Always use freshly cut sections of FFPE tissue for RNA isolation. For the first time user, we recommend to use less than 3-5 sections with thickness of 10 µm. Depending on the yield and purity obtained, it may be possible to increase the starting material.

New in this Edition:

- Proteinase K is now supplied in a liquid form eliminating the step to resuspend prior to use. Proteinase K Solution can also be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

Kit Contents

Mag-Bind® FFPE RNA (M2555)

Product Number	M2555-00	M2555-01
Preparations	5	50
Mag-Bind® Particles SC	55 µL	550 µL
RML Buffer	2 mL	30 mL
MFB Buffer	2.5 mL	12 mL
GFC Buffer	2 mL	5 mL
RNA Wash Buffer II	5 mL	12 mL
LPA Buffer	55 µL	550 µL
DNase I Digestion Buffer	0.5 mL	5 mL
DNase I	12 µL	78 µL
Proteinase K Solution (40 mg/mL)	250 µL	1.25 mL
DEPC Water	1 mL	5 mL
Instruction Booklet	1	1

Mag-Bind® FFPE RNA 96 (M2551)

Product Number	M2551-00	M2551-01	M2551-02
Preparations	1 x 96	4 x 96	20 x 96
Mag-Bind® Particles SC	2.2 mL	8.4 mL	42 mL
RML Buffer	35 mL	140 mL	700 mL
MFB Buffer	20 mL	80 mL	400 mL
GFC Buffer	10 mL	40 mL	200 mL
RNA Wash Buffer II	25 mL	100 mL	3 x 100 mL
LPA Buffer	1.1 mL	4.4 mL	22 mL
DNase I Digestion Buffer	10 mL	40 mL	200 mL
DNase I	150 µL	4 x 150 µL	20 x 150 µL
Proteinase K Solution	3 mL	12 mL	60 mL
DEPC Water	20 mL	40 mL	160 mL
Instruction Booklet	1	1	1

Preparing Reagents

Please take a few minutes to read this manual thoroughly to become familiar with the protocol before beginning the procedure. Prepare all materials required before starting to minimize RNA degradation. Wear gloves/protective goggles and take great care when working with chemicals

Dilute RNA Wash Buffer II with 100% ethanol and store at room temperature.

Kit	100% Ethanol to be Added
M2555-00	20 mL
M2555-01	48 mL

Kit	100% Ethanol to be Added
M2551-00	100 mL
M2551-01	400 mL
M2551-02	400 mL per bottle

Dilute GFC Buffer with 100% ethanol and store at room temperature.

Kit	100% Ethanol to be Added
M2555-00	4 mL
M2555-01	10 mL

Kit	100% Ethanol to be Added
M2551-00	20 mL
M2551-01	80 mL
M2551-02	400 mL

Storage and Stability

All of the Mag-Bind® FFPE RNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. DNase I should be stored at -20°C. Mag-Bind® Particles SC and LPA Buffer should be stored at 2-8°C for long-term use. Proteinase K Solution can be stored at room temperature for up to 12 months. For long-term storage, store Proteinase K Solution at 2-8°C. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in RML Buffer and MFB Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Mag-Bind® FFPE RNA Kit Protocol - 1.5 mL microcentrifuge tubes

Materials and Equipment to be Supplied by User:

- Nuclease-free 1.5 mL microcentrifuge tubes
- Centrifuge capable of 13,000 x *g*
- RNase-free filter pipette tips
- Magnetic separation device for 1.5 mL microcentrifuge tubes (MSD-02)
- Water bath or heat block capable of 55°C
- Water bath or heat block capable of 80°C
- Water bath or heat block capable of 37°C
- 100% ethanol

Before Starting:

- Prepare RNA Wash Buffer II and GFC Buffer according to the Preparing Reagents section on Page 4
- Set water bath or heat block to 55°C
- Set water bath or heat block to 80°C
- Set water bath or heat block to 37°C

1. Pipette 250 µL RML Buffer into a 1.5 mL microcentrifuge tube.

2. Cut 2-5 paraffin sample sections between 5-10 µm.

   Note: Do not use the first 2-3 sections.

3. Immediately place the sections into the 1.5 mL microcentrifuge tube. Vortex or shake for 30 seconds to mix thoroughly.

4. Centrifuge at 13,000 x *g* for 20 seconds at room temperature.

5. Incubate at 80°C for 15 minutes to melt the paraffin. Mix the sample a few times by gently shaking the tube. Make sure that the tissue sections stay submerged in the solution.

   Note: Close the cap on the tube to prevent evaporation during incubation.

6. Add 20 µL Proteinase K Solution (40 mg/mL) to each sample.

7. Incubate at 55°C for 15-30 minutes with occasional mixing. If necessary, extend the incubation to 1-3 hours or until the tissue is completely lysed.

8. Incubate at 80°C for 15 minutes.

9. Immediately centrifuge at 13,000 x *g* for 5 minutes. The paraffin will form a thin layer on top of the lysate solution.

10. Use a 1 mL pipette tip or large orifice tip to penetrate the paraffin layer, transfer 200 µL cleared lysate into a new 1.5 mL microcentrifuge tube.

11. Add 200 µL MFB Buffer, 10 µL Mag-Bind® Particles SC, and 430 µL 100% ethanol. Vortex for 20 seconds or pipet up and down 10-20 times to mix thoroughly.

    Note: If the RNA content from sample is expected low or miRNA is the target, then add 10 µL LPA Buffer.

12. Let sit at room temperature for 5-10 minutes.

13. Place the tube onto a magnetic separation device for 1.5 mL microcentrifuge tubes (MSD-02) and wait 7-10 minutes or until the Mag-Bind® Particles SC are cleared from solution.

14. Aspirate and discard the cleared supernatant.

15. Remove the tube from the magnetic separation device.

16. Add 400 µL RNA Wash Buffer II. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol prior to use. Please see instructions on Page 4.

17. Place the tube onto the magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

18. Aspirate and discard the cleared supernatant. Remove any liquid drops from tube.

19. Add 73.5 µL DNase I Digestion Buffer and 1.5 µL RNase-free DNase I to each sample. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

20. Incubate at 37°C for 15 minutes.

21. Add 225 µL GFC Buffer. Mix thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

    Note: GFC Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 4.

22. Let sit at room temperature for 3-5 minutes.

23. Place the tube onto a magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

24. Aspirate and discard the cleared supernatant.

25. Remove the tube from the magnetic separation device.

26. Add 400 µL RNA Wash Buffer II. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

27. Place the tube onto a magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

28. Aspirate and discard the cleared supernatant. Remove any liquid drops from tube.

29. Repeat Step 25-28 for a second RNA Wash Buffer II wash step.

30. Air dry the Mag-Bind® Particles SC by leaving the tubes on the magnetic separation device for 10 minutes. Remove any residual liquid with a pipette.

31. Add 30-50 µL DEPC Water. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

32. Let sit at room temperature for 5 minutes.

33. Place the tube onto a magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

34. Transfer the cleared supernatant containing purified RNA into a new nuclease-free 1.5 mL microcentrifuge tube.

35. Store the purified RNA at -80°C.

Mag-Bind® FFPE RNA Kit Protocol with Xylene - 1.5 mL microcentrifuge tubes

Note: The following protocol uses xylene to remove paraffin from the FFPE sample. Use fume hood and take proper protection during xylene extraction.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Xylene
- Nuclease-free 1.5 mL tubes
- Centrifuge capable of 13,000 x *g*
- RNase-free filter pipette tips
- Magnetic separation device for 1.5 mL tubes (MSD-02)
- Water bath or heat block capable of 55°C
- Water bath or heat block capable of 80°C
- Water bath or heat block capable of 37°C

Before Starting:

- Prepare RNA Wash Buffer II and GFC Buffer according to the Preparing Reagents section on Page 4
- Set water bath or heat block to 55°C
- Set water bath or heat block to 80°C
- Set water bath or heat block to 37°C

1. Add 1 mL xylene into a new 1.5 mL tube.

2. Cut 2-5 paraffin sample sections between 5-10 µm.

   Note: Do not use the first 2-3 sections.

3. Immediately place 2-5 sections of sample into the tube.

4. Let sit at room temperature for 2 minutes.

5. Mix thoroughly by vortexing for 20 seconds.

6. Centrifuge at 13,000 x *g* at room temperature for 5 minutes to pellet the tissue.

   Note: If the tissue does not form a tight pellet, centrifuge for an additional 3 minutes.

7. Carefully remove and discard the xylene without disturbing the pellet.

8. Add 1 mL 100% ethanol. Mix thoroughly by vortexing for 20 seconds.

9. Centrifuge at 13,000 x *g* for 5 minutes to pellet the tissue sample. The pellet should appear opaque.

10. Carefully remove and discard the ethanol. Remove any liquid drops with a pipette.

11. Repeat Steps 8-10 for a second ethanol wash step.

12. Air dry the tissue pellet for 10-20 minutes.

    Note: It is critical to completely dry the sample before the Proteinase K digestion step. Ethanol residue will effect the efficiency of the Proteinase K digestion. If a vacuum oven is available, place the tube into the vacuum oven preset at 45°C for 10-30 minutes.

13. Add 250 µL RML Buffer and 20 µL Proteinase K Solution (40 mg/mL). Resuspend the pellet by vortexing or pipetting up and down 20 times.

14. Incubate at 55°C for 15 minutes.

15. Incubate at 80°C for 15 minutes.

16. Centrifuge at 13,000 x *g* at room temperature for 5 minutes.

17. Carefully transfer 200 µL cleared supernatant into a new 1.5 mL tube.

18. Add 200 µL MFB Buffer, 20 µL Mag-Bind® Particles SC, and 430 µL 100% ethanol. Mix thoroughly by vortexing or pipetting up and down 10-20 times.

    Note: If the RNA content from sample is expected low or miRNA is the target, add 10 µL LPA Buffer.

19. Let sit at room temperature for 5-10 minutes.

20. Place the tube onto a magnetic separation device for 1.5 mL tubes (MSD-02) and wait 7-10 minutes or until the Mag-Bind® Particles SC are cleared from solution.

21. Aspirate and discard the cleared supernatant.

22. Remove the tube from the magnetic separation device.

23. Add 400 µL RNA Wash Buffer II. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol prior to use. Please see instructions on Page 4.

24. Place the tube onto the magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

25. Aspirate and discard the cleared supernatant. Remove any liquid drops from tube.

26. Add 73.5 µL DNase I Digestion Buffer and 1.5 µL Rnase-free DNase I. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

27. Incubate at 37°C for 15 minutes.

28. Add 225 µL GFC Buffer. Mix thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

    Note: GFC Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 4.

29. Let sit at room temperature for 3-5 minutes.

30. Place the tube onto a magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

31. Aspirate and discard the cleared supernatant.

32. Remove the tube from the magnetic separation device.

33. Add 400 µL RNA Wash Buffer II. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

34. Place the tube onto a magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

35. Aspirate and discard the cleared supernatant. Remove any liquid drops from tube.

36. Repeat Step 32-35 for a second RNA Wash Buffer II wash step.

37. Air dry the Mag-Bind® Particles SC by leaving the tubes on the magnetic separation device for 10 minutes. Remove any residual liquid with a pipette.

38. Add 30-50 µL DEPC Water. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

39. Let sit at room temperature for 5 minutes.

40. Place the tube onto a magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

41. Transfer the cleared supernatant containing purified RNA into a new nuclease-free 1.5 mL tube.

42. Store the purified RNA at -80°C.

Mag-Bind® FFPE RNA 96 Kit Protocol - 96-well plates

Materials and Equipment to be Supplied by User:

- Nuclease-free 1.2 mL round-well plates
- Nuclease-free microplates
- Centrifuge with swing-bucket rotor capable of 4,000 x *g*
- RNase-free filter pipette tips
- Magnetic separation device for 96-well plates (MSD-01B or MSD-01)
- Water bath or heat block capable of 55°C
- Water bath or heat block capable of 80°C
- Water bath or heat block capable of 37°C
- 100% ethanol
- Sealing film

Before Starting:

- Prepare RNA Wash Buffer II and GFC Buffer according to the Preparing Reagents section on Page 4
- Set water bath or heat block to 55°C
- Set water bath or heat block to 80°C
- Set water bath or heat block to 37°C

1. Add 250 µL RML Buffer into each well of a 1.2 mL round-well plate.

2. Cut 2-5 paraffin sample sections between 5-10 µm to be placed in each well of the 96 plate. Note: Do not use the first 2-3 sections.

3. Immediately place 2-5 sections into each well of the round-well plate.

4. Centrifuge at 4,000 x *g* at room temperature for 2 minutes.

5. Incubate at 80°C for 15 minutes to melt the paraffin. Mix the sample a few times by gently shaking the tube. Make sure that the tissue sections stay submerged in the solution.

   Note: Seal the plate with sealing film to prevent evaporation during incubation.

6. Add 25 µL Proteinase K Solution (20 mg/mL). Incubate at 55°C for 15-30 minutes with occasional mixing. If necessary, extend the incubation to 1-3 hours or until the tissue is completely lysed.

7. Incubate at 80°C for 15 minutes.

8. Immediately centrifuge at 4,000 x *g* for 5 minutes. The paraffin will form a thin layer on top of the lysate solution.

9. Use a 1 mL pipette tip or large orifice tip to penetrate the paraffin layer, transfer 200 µL cleared lysate into a new round-well plate.

10. Add 200 µL MFB Buffer, 20 µL Mag-Bind® Particles SC, and 430 µL of 100% ethanol. Mix thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

    Note: If the RNA content from sample is expected low or miRNA is the target, then add 10 µL LPA Buffer.

11. Let sit at room temperature for 5-10 minutes.

12. Place the plate onto a magnetic separation device for deep-well plates and wait 7-10 minutes or until the Mag-Bind® Particles SC are cleared from solution.

    Note: If using the MSD-01 magnetic separation device, a 500 µL processing plate (EZ960-01/02) is required for the rest of the protocol. Since the total volume of the sample is around 850 µL, this particular magnetic separation device requires the sample be transferred twice to process whole sample.

13. Aspirate and discard the cleared supernatant.

14. Remove the plate from the magnetic separation device.

15. Add 400 µL RNA Wash Buffer II. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol prior to use. Please see instructions on Page 4.

16. Place the plate onto the magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

17. Aspirate and discard the cleared supernatant. Remove any liquid drops from each well.

18. Add 73.5 µL DNase I Digestion Buffer and 1.5 µL Rnase-free DNase I. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

19. Incubate at 37°C for 15 minutes.

20. Add 225 µL GFC Buffer. Mix thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

    Note: GFC Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 4.

21. Let sit at room temperature for 3-5 minutes.

22. Place the plate onto a magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

23. Aspirate and discard the cleared supernatant.

24. Remove the plate from the magnetic separation device.

25. Add 400 µL RNA Wash Buffer II. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

26. Place the plate onto a magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

27. Aspirate and discard the cleared supernatant. Remove any liquid drops from tube.

28. Repeat Step 24-27 for a second RNA Wash Buffer II wash step.

29. Air dry the Mag-Bind® Particles SC by leaving the plate on the magnetic separation device for 10 minutes. Remove any residual liquid with a pipette.

30. Add 30-50 µL DEPC Water. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 30 seconds or pipetting up and down 30 times.

31. Let sit at room temperature for 10 minutes.

32. Place the plate onto a magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

33. Transfer the cleared supernatant containing purified RNA into a new nuclease-free 96-well microplate (not supplied) and seal with sealing film.

34. Store the purified RNA at -80°C.

Mag-Bind® FFPE RNA 96 Kit Protocol with Xylene - 96-well plates

Note: The following protocol uses xylene to remove paraffin from the FFPE sample. Use fume hood and take proper protection during xylene extraction.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Xylene
- Nuclease-free 1.2 mL round-well plates
- Nuclease-free microplates
- Centrifuge with swing-bucket rotor capable of 4,000 x *g*
- RNase-free filter pipette tips
- Magnetic separation device for 96-well plates (MSD-01B or MSD-01)
- Water bath or heat block capable of 55°C
- Water bath or heat block capable of 80°C
- Water bath or heat block capable of 37°C
- Sealing film

Before Starting:

- Prepare RNA Wash Buffer II and GFC Buffer according to the Preparing Reagents section on Page 4
- Set water bath or heat block to 55°C
- Set water bath or heat block to 80°C
- Set water bath or heat block to 37°C

1. Add 1 mL xylene into each well of a 1.2 mL round-well plate.

2. Cut 2-5 paraffin sample sections between 5-10 µm.

   Note: Do not use the first 2-3 sections.

3. Immediately place 2-5 sections each well of the 1.2 mL round-well plate.

4. Let sit at room temperature for 2 minutes.

5. Mix thoroughly by vortexing for 20 seconds.

6. Centrifuge at 4,000 x *g* at room temperature for 5 minutes to pellet the tissue.

   Note: If the tissue does not form a tight pellet, centrifuge for an additional 3 minutes.

7. Carefully remove and discard the xylene without disturbing the pellets.

8. Add 1 mL 100% ethanol to each well. Mix thoroughly by vortexing for 20 seconds.

9. Centrifuge at 4,000 x *g* for 5 minutes to pellet the tissue samples. The pellets should appear opaque.

10. Carefully remove and discard the ethanol. Remove any liquid drops with a pipette.

11. Repeat Steps 8-10 for a second ethanol wash step.

12. Air dry the tissue pellet for 10-20 minutes.

    Note: It is critical to completely dry the sample before the Proteinase K digestion step. Ethanol residue will effect the efficiency of the Proteinase K digestion. If a vacuum oven is available, place the tube into the vacuum oven preset at 45°C for 10-30 minutes.

13. Add 250 µL RML Buffer and 25 µL Proteinase K Solution (20 mg/mL). Resuspend the pellet by vortexing or pipetting up and down 20 times.

14. Incubate at 55°C for 15 minutes.

15. Incubate at 80°C for 15 minutes.

16. Centrifuge at 4,000 x *g* at room temperature for 5 minutes.

17. Carefully transfer 200 µL cleared supernatant into a new round-well plate.

18. Add 200 µL MFB Buffer, 20 µL Mag-Bind® Particles SC, and 430 µL 100% ethanol. Mix thoroughly by vortexing or pipetting up and down 10-20 times.

    Note: If the RNA content from sample is expected low or miRNA is the target, add 10 µL LPA Buffer.

19. Let sit at room temperature for 5-10 minutes.

20. Place the plate onto a magnetic separation device for deep-well plates and wait 7-10 minutes or until the Mag-Bind® Particles SC are cleared from solution.

    Note: If using the MSD-01 magnetic separation device, a 500 µL processing plate (EZ960-01/02) is required for the rest of the protocol. Since the total volume of the sample is around 850 µL, this particular magnetic separation device requires the sample be transferred twice to process whole sample.

21. Aspirate and discard the cleared supernatant.

22. Remove the plate from the magnetic separation device.

23. Add 400 µL RNA Wash Buffer II. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol prior to use. Please see instructions on Page 4.

24. Place the plate onto the magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

25. Aspirate and discard the cleared supernatant. Remove any liquid drops from each well.

26. Add 73.5 µL DNase I Digestion Buffer and 1.5 µL Rnase-free DNase I. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

27. Incubate at 37°C for 15 minutes.

28. Add 225 µL GFC Buffer. Mix thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

    Note: GFC Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 4.

29. Let sit at room temperature for 3-5 minutes.

30. Place the plate onto a magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

31. Aspirate and discard the cleared supernatant.

32. Remove the plate from the magnetic separation device.

33. Add 400 µL RNA Wash Buffer II. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 20 seconds or pipetting up and down 10-20 times.

34. Place the plate onto a magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

35. Aspirate and discard the cleared supernatant. Remove any liquid drops from the wells.

36. Repeat Step 32-35 for a second RNA Wash Buffer II wash step.

37. Air dry the Mag-Bind® Particles SC by leaving the plate on the magnetic separation device for 10 minutes. Remove any residual liquid with a pipette.

38. Add 30-50 µL DEPC Water. Resuspend the Mag-Bind® Particles SC thoroughly by vortexing for 30 seconds or pipetting up and down 30 times.

39. Let sit at room temperature for 10 minutes.

40. Place the plate onto a magnet separation device to magnetize the Mag-Bind® Particles SC. Wait 3-5 minutes or until all the Mag-Bind® Particles SC are cleared from solution.

41. Transfer the cleared supernatant containing purified RNA into a new nuclease-free 96-well microplate (not supplied) and seal with sealing film.

42. Store the purified RNA at -80°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact our technical support staff, toll free, at **800-832-8896.**

Possible Problems and Suggestions

Troubleshooting Guide		
Problem	**Likely Cause**	**Suggestions**
Low RNA yields	Incomplete re-suspension of magnetic particles	Resuspend the magnetic particles by vortexing before use.
	RNA degraded during sample storage	Make sure the sample is properly stored and make sure the samples are processed immediately after collection or removal from storage.
	RNA Wash Buffer II was not prepared correctly	Prepare RNA Wash Buffer II by adding ethanol according to the instructions.
	Loss of magnetic beads during operation	Increase the beads collection time.
	GFC Buffer not diluted with ethanol	Prepare GFC Buffer as instructed on the label.
	RNA Wash Buffer II was not prepared correctly	Prepare RNA Wash Buffer II by adding ethanol according to the instructions.
Problem with downstream application	Degraded RNA	During incubation at 37°C, do not incubate sample over 15 minutes.
Carryover of the magnetic beads in the elution	Carryover the magnetic beads in the eluted RNA will not effect downstream applications.	To remove the carryover magnetic particles from the eluted RNA, simply magnetize the magnetic particles and carefully transfer the RNA eluate to a new plate.