omega BIO-TEK

innovations in nucleic acid isolation

Product Manual

Mag-Bind® Universal Pathogen 96 Kit

M4029-00	1 x 96 preps
M4029-01	4 x 96 preps

Mag-Bind® Universal Pathogen Core Kit

M4030-00	1 x 96 preps
M4030-01	4 x 96 preps

Manual Date: December 2021
Manual Revision: v6.0

For Research Use Only

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Mag-Bind® Universal Pathogen 96 Kit
Mag-Bind® Universal Pathogen Core Kit

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Introduction

The Mag-Bind® Universal Pathogen 96 Kit allows rapid and reliable isolation of high-quality host genomic DNA, gram positive and negative bacterial DNA , fungal spore DNA, and viral DNA and viral RNA from tissue, urine, serum, and fecal samples. The system allows for automation after sample lysis via Hamilton STAR™, Thermo KingFisher™ Flex, Applied Biosystems® MagMAX™, Qiagen BioSprint, and other liquid handling instruments.

The system combines the Mag-Bind® technology with RBB Buffer to eliminate PCR inhibiting compounds within the samples. Purified DNA is suitable for NGS, PCR, restriction digestion, and hybridization applications. There are no organic extractions thus reducing plastic waste and hands-on time allowing multiple samples to be processed in parallel.

Mag-Bind® Universal Pathogen 96 Kit (M4029) includes Disruptor Plate C Plus that employs bead beating technology for mechanical homogenization of the sample. For customers who prefer to not employ mechanical homogenization or would like to follow their own homogenization method, we offer Mag-Bind® Universal Pathogen Core Kit (M4030) that does not contain Disruptor Plate C Plus.

New in this Edition:

November 2021

- This manual has been modified to include information for M4030-00 and M4030-01 kits.

May 2019

- SPM Wash Buffer has been renamed SPM Buffer. This is a name change only. The formulation has not changed.
- XP2 Buffer has been renamed XP2 Binding Buffer. This is a name change only. The formulation has not changed.

December 2017

- E-Z 96 Disruptor Plate C Plus has replaced E-Z 96 Disruptor Plus Plate to improve overall performance and prevent leakage.

Kit Contents

Product Number	M4029-00	M4029-01
Purifications	1 x 96 preps	4 x 96 preps
E-Z 96 Disruptor Plate C Plus*	1	4
Caps for Racked Microtubes*	13	52
SLX-Mlus Buffer	60 mL	240 mL
DS Buffer	8 mL	30 mL
PCP Buffer	25 mL	100 mL
XP2 Binding Buffer	40 mL	160 mL
RBB Buffer	40 mL	160 mL
VHB Buffer	88 mL	3 x 88 mL
SPM Buffer	30 mL	4 x 30 mL
Elution Buffer	30 mL	60 mL
Proteinase K Solution	2.2 mL	9 mL
Mag-Bind® Particles RQ	2.2 mL	9 mL
User Manual	✓	✓

* These 2 components are not included in M4030-00 and M4030-01 kits.

Product Number	M4030-00	M4030-01
Purifications	1 x 96 preps	4 x 96 preps
SLX-Mlus Buffer	60 mL	240 mL
DS Buffer	8 mL	30 mL
PCP Buffer	25 mL	100 mL
XP2 Binding Buffer	40 mL	160 mL
RBB Buffer	40 mL	160 mL
VHB Buffer	88 mL	3 x 88 mL
SPM Buffer	30 mL	4 x 30 mL
Elution Buffer	30 mL	60 mL
Proteinase K Solution	2.2 mL	9 mL
Mag-Bind® Particles RQ	2.2 mL	9 mL
User Manual	✓	✓

1. Dilute SPM Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M4029-00 **M4030-00**	70 mL
M4029-01 **M4030-01**	70 mL per bottle

2. Dilute VHB Buffer with 100% ethanol follows and store at room temperature.

Kit	100% Ethanol to be Added
M4029-00 **M4030-00**	112 mL
M4029-01 **M4030-01**	112 mL per bottle

Storage and Stability

All of the Mag-Bind® Universal Pathogen 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles RQ must be stored at 2-8°C. 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 some of the buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Samples

If using Disruptor Plate C Plus (M4029-00 or M4029-01 kit), follow protocols as written starting on Page 7.

If no homogenization is reguired, it is recommended to use M4030-00 or M4030-01 kit that does not contain Disruptor Plate C Plus. Follow the instructions below for sample preparation.

1. No homogenization method for Tissue Samples:

    a. Prepare a mastermix of SLX-Mlus Buffer, Proteinase K Solution, and DS Buffer according to the table below:

Component	Amount per Prep	Total Amount per 96-well Plate
SLX-Mlus Buffer	525 µL	55.4 mL*
DS Buffer	53 µL	5.6 mL*
Proteinase K Solution	20 µL	2.1 mL*

*10% excess volume has been calculated for a 96-well plate.

    b. Add 600 µL SLX-Mlus Buffer/DS Buffer/Proteinase K Solution mastermix to each well of a plate containing your sample.
    c. Continue to Step 9 on Page 8 for Tissue Protocol.

2. No homogenization method for Serum & Stool or Urine Samples:

    a. Prepare a mastermix of SLX-Mlus Buffer, Proteinase K Solution, and DS Buffer according to the table below:

Component	Amount per Prep	Total Amount per 96-well Plate
SLX-Mlus Buffer	275 µL	29.0 mL*
DS Buffer	50 µL	5.28 mL*
Proteinase K Solution	20 µL	2.1 mL*

*10% excess volume has been calculated for a 96-well plate.

    b. Add 350 µL SLX-Mlus Buffer/DS/Proteinase K Solution mastermix to each well of a plate containing your sample.
    c. Continue to Step 9 on Page 12 for Serum & Stool Protocol or Step 9 on Page 16 for Urine Protocol.

3. Customer modified homogenization method (does not use Disruptor Plate C Plus):

   a. Tissue Samples:
      i. Add 525 µL SLX-Mlus Buffer and perform homogenization method of choice.
      ii. Add 53 µL DS Buffer and 20 µL Proteinase K Solution.
      iii. Continue to Step 9 on Page 8 for Tissue Protocol.

   b. Serum & Stool or Urine Samples:
      i. Add 275 µL SLX-Mlus Buffer and perfom homogenization method of choice.
      ii. Add 50 µL DS Buffer and 20 µL Proteinase K Solution.
      iii. Continue to Step 9 on Page 12 for Serum & Stool Protocol or Step 9 on Page 16 for Urine Protocol.

Mag-Bind® Universal Pathogen 96 Kit - Tissue Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 3,500*g* with adapter for 96-well plates
- Magnetic Separation Device (Recommend Cat# Alpaqua® 96M A000250)
- Incubator capable of 70°C
- 96-well plates with a capacity of at least 1.7 mL (Recommend Nunc 278752) and compatible with the Magnetic Separation Device
- 96-well microplates for DNA storage
- Vortexer
- 100% ethanol
- Nuclease-free water
- Optional: Mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Omni's Bead Ruptor 96

Before Starting:

- Prepare VHB Buffer and SPM Buffer according to the "Preparing Reagents" section on Page 4.
- Set an incubator to 70°C.
- Heat Elution Buffer to 70°C.

Important: Please see "Preparing Samples" section on Page 5 for instructions when using M4030-00 or M4030-01 kit.

1. Briefly spin the E-Z 96 Disruptor Plate C Plus to remove any glass beads from the walls of the wells. Uncap the E-Z 96 Disruptor Plate C Plus and save the caps for use in Step 3.

2. Add 25-30 mg tissue.

3. Add 525 µL SLX-Mlus Buffer. Seal the E-Z 96 Disruptor Plate C Plus with the caps removed in Step 1.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Omni's Bead Ruptor 96, should be used.

   Note: Depending on the sample amount and type, the amount of SLX-Mlus Buffer may need to be adjusted so that 300 µL can be recovered after Step 5.

5. Centrifuge at 1,000-2,000*g* for 60 seconds at room temperature.

6. Remove and discard the caps from the E-Z 96 Disruptor Plate C Plus.

7. Add 53µL DS Buffer and 20 µL Proteinase K Solution.

8. Seal the E-Z 96 Disruptor Plate C Plus with new Caps for Racked Microtubes.

9. Vortex for 60 seconds to mix thoroughly.

10. Incubate at 70°C for 15 minutes. Mix once during incubation.

11. Centrifuge at 3,500*g* for 10 minutes.

12. Transfer 300 µL cleared supernatant to a 96-well deep-well plate (1.7 mL) compatible with the Magnetic Separation Device used.

    Note: Do not transfer any debris as it can reduce yield and purity.

13. Add 300 µL XP2 Binding Buffer, 300 µL RBB Buffer, and 20 µL Mag-Bind® Particles RQ. Vortex to mix thoroughly or pipet up and down 20 times.

    Note: Mag-Bind® Particles RQ and XP2 Binding Buffer can be prepared as a mastermix prior to use. Prepare only what is needed. Tip mixing is recommended for automated protocols for best yield.

14. Let sit at room temperature for 10 minutes.

15. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

16. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

17. Remove the plate containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.

18. Add 600 µL VHB Buffer. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

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

19. Let sit at room temperature for 2 minutes.

20. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

21. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

22. Remove the plate containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.

23. Repeat Steps 18-22 once for a second VHB Wash step.

24. Add 600 µL SPM Buffer. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

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

25. Let sit at room temperature for 2 minutes.

26. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

27. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

28. Leave the plate on the Magnetic Separation Device.

29. Select one of the following ethanol removal steps:

    A. Leave the plate on the Magnetic Separation Device. Wait 1 minute. Remove residual liquid with a pipettor. Dry the Mag-Bind® Particles RQ for an additional 10 minutes. Continue to Step 30.

    OR

    B. Add 500 µL nuclease-free water (not provided). Immediately aspirate the nuclease-free water. Do not let the samples stay in contact with the nuclease-free water for more than 60 seconds. Continue to Step 30.

    Note: Yields may be compromised when using option B. If using an automated platform, use the maximum volume the tips will allow up to 600 µL.

30. Add 50-100 µL Elution Buffer heated to 70°C. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

31. Let sit at room temperature for 5 minutes.

32. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

33. Transfer the cleared supernatant containing purified DNA to a clean 96-well microplate. Store the DNA at -20°C.

Mag-Bind® Universal Pathogen 96 Kit - Serum & Stool Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 3,500*g* with adapter for 96-well plates
- Magnetic Separation Device (Recommend Cat# Alpaqua® 96M A000250)
- Incubator capable of 70°C
- 96-well plates with a capacity of at least 1.7 mL (Recommend Nunc 278752) and compatible with the Magnetic Separation Device
- 96-well microplates for DNA storage
- Vortexer
- 1X PBS
- 100% ethanol
- Nuclease-free water
- Optional: Mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Omni's Bead Ruptor 96

Before Starting:

- Prepare VHB Buffer and SPM Buffer according to the "Preparing Reagents" section on Page 4.
- Set an incubator to 70°C.
- Heat Elution Buffer to 70°C.

Important: Please see "Preparing Samples" section on Page 5 for instructions when using M4030-00 or M4030-01 kit.

1. Briefly spin the E-Z 96 Disruptor Plate C Plus to remove any glass beads from the walls of the wells. Uncap the E-Z 96 Disruptor Plate C Plus and save the caps for use in Step 3.

2. Add 250 µL serum or stool samples. If stool sample is solid, resuspend to 10% wgt/volume in PBS before starting.

3. Add 275 µL SLX-Mlus Buffer. Seal the E-Z 96 Disruptor Plate C Plus with the caps removed in Step 1.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Omni's Bead Ruptor 96, should be used.

   Note: Depending on the sample amount and type, the amount of SLX-Mlus Buffer may need to be adjusted so that 300 µL can be recovered after Step 5.

5. Centrifuge at 1,000-2,000*g* for 60 seconds at room temperature.

6. Remove and discard the caps from the E-Z 96 Disruptor Plate C Plus.

7. Add 50 µL DS Buffer and 20 µL Proteinase K Solution.

8. Seal the E-Z 96 Disruptor Plate C Plus with new Caps for Racked Microtubes.

9. Vortex for 60 seconds to mix thoroughly.

10. Incubate at 70°C for 15 minutes. Mix once during incubation.

11. Centrifuge at 3,500*g* for 10 minutes.

12. Transfer 300 µL cleared supernatant to a 96-well deep-well plate (1.7 mL) compatible with the Magnetic Separation Device used.

    Note: Do not transfer any debris as it can reduce yield and purity.

13. Add 300 µL XP2 Binding Buffer, 300 µL RBB Buffer, and 20 µL Mag-Bind® Particles RQ. Vortex to mix thoroughly or pipet up and down 20 times.

    Note: Mag-Bind® Particles RQ and XP2 Binding Buffer can be prepared as a mastermix prior to use. Prepare only what is needed. Tip mixing is recommended for automated protocols for best yield.

14. Let sit at room temperature for 10 minutes.

15. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

16. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

17. Remove the plate containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.

18. Add 600 µL VHB Buffer. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

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

19. Let sit at room temperature for 2 minutes.

20. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

21. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

22. Remove the plate containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.

23. Repeat Steps 18-22 once for a second VHB Wash step.

24. Add 600 µL SPM Buffer. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

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

25. Let sit at room temperature for 2 minutes.

26. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

27. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

28. Leave the plate on the Magnetic Separation Device.

29. Select one of the following ethanol removal steps:

    A. Leave the plate on the Magnetic Separation Device. Wait 1 minute. Remove residual liquid with a pipettor. Dry the Mag-Bind® Particles RQ for an additional 10 minutes. Continue to Step 30.

    OR

    B. Add 500 µL nuclease-free water (not provided). Immediately aspirate the nuclease-free water. Do not let the samples stay in contact with the nuclease-free water for more than 60 seconds. Continue to Step 30.

    Note: Yields may be compromised when using option B. If using an automated platform, use the maximum volume the tips will allow up to 600 µL.

30. Add 50-100 µL Elution Buffer heated to 70°C. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

31. Let sit at room temperature for 5 minutes.

32. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

33. Transfer the cleared supernatant containing purified DNA to a clean 96-well microplate. Store the DNA at -20°C.

Mag-Bind® Universal Pathogen 96 Kit - Urine Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 3,500*g* with adapter for 96-well plates
- Magnetic Separation Device (Recommend Cat# Alpaqua® 96M A000250)
- Incubator capable of 70°C
- 96-well plates with a capacity of at least 1.7 mL (Recommend Nunc 278752) and compatible with the Magnetic Separation Device
- 96-well microplates for DNA storage
- Vortexer
- Ice bucket
- 100% ethanol
- Nuclease-free water
- Optional: Mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Omni's Bead Ruptor 96

Before Starting:

- Prepare VHB Buffer and SPM Buffer according to the "Preparing Reagents" section on Page 4.
- Set an incubator to 70°C.
- Heat Elution Buffer to 70°C
- Prepare an ice Bucket

Important: Please see "Preparing Samples" section on Page 5 for instructions when using M4030-00 or M4030-01 kit.

1. Briefly spin the E-Z 96 Disruptor Plate C Plus to remove any glass beads from the walls of the wells. Uncap the E-Z 96 Disruptor Plate C Plus and save the caps for use in Step 3.

2. Add 250 µL urine sample.

3. Add 275 µL SLX-Mlus Buffer. Seal the E-Z 96 Disruptor Plate C Plus with the caps removed in Step 1.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Omni's Bead Ruptor 96, should be used.

   Note: Depending on the sample amount and type, the amount of SLX-Mlus Buffer may need to be adjusted so that 300 µL can be recovered after Step 5.

5. Centrifuge at 1,000-2,000*g* for 60 seconds at room temperature.

6. Remove and discard the caps from the E-Z 96 Disruptor Plate C Plus.

7. Add 50 µL DS Buffer and 20 µL Proteinase K Solution.

8. Seal the E-Z 96 Disruptor Plate C Plus with new Caps for Racked Microtubes.

9. Vortex for 60 seconds to mix thoroughly.

10. Incubate at 70°C for 15 minutes. Mix once during incubation.

11. Add 200 µL PCP Buffer. Place the plate on ice for 5 minutes.

12. Centrifuge at 3,500*g* for 10 minutes.

13. Transfer 300 µL cleared supernatant to a 96-well deep-well plate (1.2 mL) compatible with the Magnetic Separation Device used.

    Note: Do not transfer any debris as it can reduce yields and purity.

14. Add 300 µL XP2 Binding Buffer, 300 µL RBB Buffer, and 20 µL Mag-Bind® Particles RQ. Vortex to mix thoroughly or pipet up and down 20 times.

    Note: Mag-Bind® Particles RQ and XP2 Binding Buffer can be prepared as a mastermix prior to use. Prepare only what is needed. Tip mixing is recommended for automated protocols for best yield.

15. Let sit at room temperature for 10 minutes.

16. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

17. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

18. Remove the plate containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.

19. Add 600 µL VHB Buffer. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

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

20. Let sit at room temperature for 2 minutes.

21. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

22. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

23. Remove the plate containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.

24. Repeat Steps 19-23 once for a second VHB Wash step.

25. Add 600 µL SPM Buffer. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

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

26. Let sit at room temperature for 2 minutes.

27. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

28. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

29. Leave the plate on the Magnetic Separation Device.

30. Select one of the following ethanol removal steps:

    A. Leave the plate on the Magnetic Separation Device. Wait 1 minute. Remove residual liquid with a pipettor. Dry the Mag-Bind® Particles RQ for an additional 10 minutes. Continue to Step 31.

    OR

    B. Add 500 µL nuclease-free water (not provided). Immediately aspirate the nuclease-free water. Do not let the samples stay in contact with the nuclease-free water for more than 60 seconds. Continue to Step 31.

    Note: Yields may be compromised when using option B. If using an automated platform, use the maximum volume the tips will allow up to 600 µL.

31. Add 50-100 µL Elution Buffer heated to 70°C. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

32. Let sit at room temperature for 5 minutes.

33. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

34. Transfer the cleared supernatant containing purified DNA to a clean 96-well microplate. Store the DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
A_{260}/A_{230} ratio is low	Salt contamination	• Repeat the DNA isolation with a new sample. • Extend the incubation time with VHB Buffer. • Wash the Mag-Bind® Particles RQ with ethanol.
A_{260}/A_{280} ratio is high	RNA contamination	The protocol does not remove RNA. If desired, add 5 µL RNase A (25 mg/mL) after lysate is cleared and before binding buffers are added. Let sit at room temperature for 5 minutes.
Low DNA Yield or no DNA Yield	Poor homogenization of sample	Repeat the DNA isolation with a new sample, be sure to mix the sample with SLX-Mlus Buffer thoroughly. Use a commercial homogenizer if possible.
	DNA washed off	Make sure VHB Buffer and SPM Buffer are mixed with ethanol.
	Extended water wash	Make sure that water wash step does not exceed 60 seconds and the Mag-Bind® Particles RQ are not resuspended.
	Mag-Bind® Particles RQ lost in process	After water is added during wash step Mag-Bind® Particles RQ will go into solution. Avoid disturbing the Mag-Bind® Particles RQ during aspiration and extend magnetization time, if required.
Problems in downstream applications	BSA not added to PCR mixture	Add BSA to a final concentration of 0.1 µg/mL to the PCR mixture.
	Too much DNA inhibits PCR reactions	Dilute the DNA eluate used in the downstream application if possible.
	Non-specific bands in downstream PCR	Use hot-start Taq polymerase mixture.
Problems in downstream applications	Inhibitory substance in the eluted DNA	Check the A_{260}/A_{230} ratio. Dilute the elute to 1:50 if necessary.

Notices & Disclaimers

For European Union Use.

RBB Buffer contains Triton X-100, 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol (CAS 9002-93-1), a substance included in the European Authorisation list (Annex XIV) of REACH Regulation (EC) No 1907/2006. Substances and mixtures used for the purpose of Scientific Research and Development (SR&D) are exempt from authorization requirements if used below 1 tonne per year in volume.

Scientific Research and Development includes experimental research or analytical activities at a laboratory scale such as synthesis and testing of applications of chemicals, release tests, etc. as well as the use of the substance in monitoring and routine quality control or *in vitro* diagnostics.

For more purification solutions, visit **www.omegabiotek.com**

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