

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: April 2024
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For Research Use Only

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.

Important:

1. If automating this procedure on a liquid handler or a magnetic processor, please contact your Omega Bio-tek representative for instrument-specific instructions.
2. Kits include enough reagents for the specified number of preparations plus an additional 10% overage to ensure there is sufficient volume. Please be aware that the actual number of preparations may be lower due to pre-aliquoting of reagents, processing partial plates, and automation platform used etc. Additional reagents are available for purchase separately. Please visit the product page at www.omegabiotek.com or contact your Omega Bio-tek representative for more details and ordering information.

New in this Edition:

April 2024

- Addition of Warnings and Safety Information

October 2022

- An important statement is included clarifying how the actual number of preparations is dependent on various factors and may be lower than the number of preparations specified with the kit.
- Urine Protocol has been updated to reflect additional Caps for Racked Microtubes that are needed. These can be purchased separately as a bag of 50 (Cat# CRMT-50).

Introduction

November 2021

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

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	✓	✓

Preparing Reagents

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. Store all other components at room temperature and away from bright light. 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.

Warnings and Safety Information

Warnings

This kit is for research use only.

Please read all instructions carefully before using the kit.

Decontaminate and dispose of all potentially infectious materials in accordance with applicable local, state, and national regulations. Please refer to safety data sheets (SDSs) for information on disposal of different components included in this kit.

Safety Information

All chemicals and biological materials are potentially hazardous. Biological samples such as plasma, serum, tissues, body fluids, blood etc. are potentially infectious and must be treated as biohazardous materials. Conduct all work in properly equipped facilities following universal precautions and using appropriate personal safety equipment such as disposable gloves, lab coats, safety glasses etc. as required by policies and procedures outlined by your facility. Please refer to safety data sheets (SDSs) for information on safe handling, transport and disposal of different components included in this kit. SDSs are made available in PDF format on the product page at www.omegabiotek.com. Discard all waste in accordance with the local safety regulations.

Some of the buffers included in the product contain guanidine-based chaotropic agents, which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions to guanidine-containing waste. Please access the SDSs online for detailed information on the reagents.

Preparing Samples

If using Disruptor Plate C Plus (M4029-00 or M4029-01 kit), follow protocols as written starting on Page 9.

If no homogenization is required, 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 μ L	55.4 mL*
DS Buffer	53 μ L	5.6 mL*
Proteinase K Solution	20 μ L	2.1 mL*

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

- b. Add 600 μ L 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 10 for Tissue Protocol.

2. No homogenization method for Serum & Stool 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 μ L	29.0 mL*
DS Buffer	50 μ L	5.28 mL*
Proteinase K Solution	20 μ L	2.1 mL*

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

- b. Add 350 μ L SLX-Mlus Buffer/DS/Proteinase K Solution mastermix to each well of a plate containing your sample.
- c. Continue to Step 9 on Page 14 for Serum & Stool Protocol.

Preparing Samples

3. No homogenization method for 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 μ L	29.0 mL*
DS Buffer	50 μ L	5.28 mL*
Proteinase K Solution	20 μ L	2.1 mL*

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

- b. Add 350 μ L SLX-Mlus Buffer/DS/Proteinase K Solution mastermix to each well of a plate containing your sample.
- c. Vortex for 60 seconds to mix thoroughly.
- d. Incubate at 70°C for 15 minutes. Mix once during incubation.
- e. Add 200 μ L PCP Buffer.
- f. Vortex for 60 seconds to mix thoroughly.
- g. Continue to Step 13 on Page 18 for Urine Protocol.

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

- a. Tissue Samples:
 - i. Add 525 μ L SLX-Mlus Buffer and perform homogenization method of choice.
 - ii. Add 53 μ L DS Buffer and 20 μ L Proteinase K Solution.
 - iii. Continue to Step 9 on Page 10 for Tissue Protocol.
- b. Serum & Stool Samples
 - i. Add 275 μ L SLX-Mlus Buffer and perform homogenization method of choice.
 - ii. Add 50 μ L DS Buffer and 20 μ L Proteinase K Solution.
 - iii. Continue to Step 9 on Page 14 for Serum & Stool Protocol or Step 9 on Page 18 for Urine Protocol
- c. Urine Samples:
 - i. Add 275 μ L SLX-Mlus Buffer and perform homogenization method of choice.
 - ii. Add 50 μ L DS Buffer and 20 μ L Proteinase K Solution.
 - iii. Vortex for 60 seconds to mix thoroughly.
 - iv. Incubate at 70°C for 15 minutes. Mix once during incubation.
 - v. Add 200 μ L PCP Buffer.
 - vi. Vortex for 60 seconds to mix thoroughly.
 - vii. Continue to Step 13 on Page 18 for Urine Protocol.

Mag-Bind® Universal Pathogen 96 Kit Tissue Protocol

Mag-Bind® Universal Pathogen 96 Kit - Tissue Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 3,500g 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 5.
- Set an incubator to 70°C.
- Heat Elution Buffer to 70°C.

Important: Please see "Preparing Samples" section on Page 7 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.

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Tissue Protocol

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 1,000-2,000g 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 3,500g 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.

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Tissue Protocol

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 μ L 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 5 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 μ L 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 5 for instructions.

Mag-Bind® Universal Pathogen 96 Kit

Tissue Protocol

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

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

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 3,500g 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 5.
- Set an incubator to 70°C.
- Heat Elution Buffer to 70°C.

Important: Please see "Preparing Samples" section on Page 7 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.

Mag-Bind® Universal Pathogen 96 Kit

Serum & Stool Protocol

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 1,000-2,000g 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 3,500g 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.

Mag-Bind® Universal Pathogen 96 Kit

Serum & Stool Protocol

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 μ L 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 5 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 μ L 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 5 for instructions.

Mag-Bind® Universal Pathogen 96 Kit

Serum & Stool Protocol

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

Mag-Bind® Universal Pathogen 96 Kit - Urine Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 3,500g 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
- Caps for Racked Microtubes (Cat# CRMT-50)
- 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 5.
- Set an incubator to 70°C.
- Heat Elution Buffer to 70°C
- Prepare an ice Bucket
- An additional set of Caps for Racked Microtubes is needed for this protocol. These can be purchased separately, Cat# CRMT-50.

Important: Please see "Preparing Samples" section on Page 7 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.

Mag-Bind® Universal Pathogen 96 Kit

Urine Protocol

3. Add 275 μ L 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 μ L can be recovered after Step 5.

5. Centrifuge at 1,000-2,000g for 60 seconds at room temperature.
6. Remove and discard the caps from the E-Z 96 Disruptor Plate C Plus.
7. Add 50 μ L DS Buffer and 20 μ L 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. Remove and discard the caps from the E-Z 96 Disruptor Plate C Plus.
12. Add 200 μ L PCP Buffer. Seal the E-Z 96 Disruptor Plate C Plus with new Caps for Racked Microtubes.

Note: An additional set of Caps for Racked Microtubes is needed for this protocol. These can be purchased separately, Cat# CRMT-50.
13. Place the plate on ice for 5 minutes.

Mag-Bind® Universal Pathogen 96 Kit

Urine Protocol

14. Centrifuge at 3,500g for 10 minutes.
15. 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.

16. 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.

17. Let sit at room temperature for 10 minutes.
18. 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.
19. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.
20. Remove the plate containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.
21. 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 5 for instructions.

22. Let sit at room temperature for 2 minutes.
23. 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.

Mag-Bind® Universal Pathogen 96 Kit

Urine Protocol

24. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.
25. Remove the plate containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.
26. Repeat Steps 19-23 once for a second VHB Wash step.
27. 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 5 for instructions

28. Let sit at room temperature for 2 minutes.
29. 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.
30. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.
31. Leave the plate on the Magnetic Separation Device.
32. 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 33.

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 33.

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.

Mag-Bind® Universal Pathogen 96 Kit

Urine Protocol

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

34. Let sit at room temperature for 5 minutes.

35. 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.

36. 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	<ul style="list-style-type: none"> 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 μ L 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 μ g/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.

Notes:



For more purification solutions, visit www.omegabiotek.com

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