MAGBIO

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# HighPrep™ Pathogen Maxi Kit

Manual Revision v1.1
Catalog Nos. HPPM-D16, HPPM-D96, HPPM-D96X4

- Genomic DNA isolation from various pathogens from tissue, urine, serum, and fecal samples
- Magnetic beads based chemistry

# PROTOCOL

## Contents



**For Research Use Only. Not for use in diagnostic procedures.**

Information in this document is subject to change without notice.

## Product Description

MagBio Pathogen Maxi is paramagnetic bead-based kit, it is designed for efficient isolation of high-quality genomic DNA from various pathogens including gram positive and negative bacterial DNA, fungal spore DNA, and viral DNA and RNA from tissue, urine, serum, and fecal samples.
The kit's utilization method is adaptable on many major liquid handling workstations for high throughput processing, and will generate genomic DNA, suitable for PCR, restriction digestion, and hybridization applications.

## Process

The HighPrep™ Pathogen Maxi Kit uses a simple 3 step procedure: Lyse+Bind-Wash-Elute. Samples are lysed and DNA binds to the MAG-RQ1 magnetic beads in one step. Utilizing a magnetic separation device, the bound genomic DNA is separated from the solution and is washed. The final step is elution of high quality genomic DNA from the magnetic beads.

## Kit Contents and Storage

| HighPrep™ Pathogen Maxi Kit Catalog No. | HPPM-D16 | HPPM-D96 | HPPM-D384 | STORAGE |
|---|---|---|---|---|
| **Number of Preps** | **16** | **96** | **384** | |
| PL Buffer | 10 ml | 60 ml | 240 ml | 15-25°C |
| PB Buffer | 1.5 ml | 8 ml | 30 ml | 15-25°C |
| PP Buffer | 4.5 ml | 25 ml | 100 ml | 15-25°C |
| PBG Buffer | 7 ml | 40 ml | 160 ml | 15-25°C |
| PBB Buffer | 7 ml | 40 ml | 160 ml | 15-25°C |
| PBH Buffer | 15 ml | 88 ml | 3 x 88 ml | 15-25°C |
| MQ Wash Buffer | 5 ml | 30 ml | 4 x 30 ml | 15-25°C |
| MB Elution Buffer | 2.5 ml | 15 ml | 50 ml | 15-25°C |
| Pro K Solution [2] | 380 µl | 2.2 ml | 9 ml | 2-8°C |
| MAG-RQ1 Particles | 380 µl | 2.2 ml | 9 ml | 2-8°C |

[1] Ethanol must be added prior to use. See Preparation of Reagents

## Stability

All components are stable for 12 months when stored accordingly.
[2] Pro K Solution comes in a ready to use solution. Component is stable for 1 year when stored at 15-25°C. For storage longer than 1 year, storage at 2-8°C is recommended.

## Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Resource" tab when viewing the product kit.

## Preparation of Reagents

Prepare the following components for each kit before use:

| Catalog No. | Component | Add 100% Ethanol | Storage |
|---|---|---|---|
| **HPPM-D16** | MQ Wash Buffer | 17.5 ml | Room Temp 15-25°C |
| **HPPM-D16** | PBH Buffer | 28 ml | Room Temp 15-25°C |

Components are stable for 1 year when stored closed at room temperature

| Catalog No. | Component | Add 100% Ethanol | Storage |
|---|---|---|---|
| **HPPM-D96** | MQ Wash Buffer | 70 ml | Room Temp 15-25°C |
| **HPPM-D96** | PBH Buffer | 112 ml | Room Temp 15-25°C |

Components are stable for 1 year when stored closed at room temperature

| Catalog No. | Component | Add 100% Ethanol | Storage |
|---|---|---|---|
| **HPPM-D384** | MQ Wash Buffer | 70 ml per Bottle | Room Temp 15-25°C |
| **HPPM-D384** | PBH Buffer | 112 ml per Bottle | Room Temp 15-25°C |

Components are stable for 1 year when stored closed at room temperature

# HighPrep™ Pathogen Maxi Kit : Tissue Protocol

***The following protocol can be applied for saliva samples.***

## Materials and Equipment to be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ Centrifuge capable of at least 3,500 x g
- ☐ Magnetic Separation Device (MBMS-10)
- ☐ Incubator capable of 65°C
- ☐ 1.5ml tubes
- ☐ Vortexer
- ☐ 100% ethanol
- ☐ Nuclease Free Water

## Things to do before starting

- ☐ Prepare PBH Buffer and MQ Wash Buffer according to the "Preparing Reagents" section on Page 2.
- ☐ Set an incubator to 65°C.
- ☐ Heat Elution Buffer to 70°C

## Protocol

1. **Add 25-30 mg tissue to each 1.5mL tube after mincing the tissue.**
2. **Add 525 µL PL Buffer to each sample.**
3. **Add 53µL PB Buffer and 20 µL Proteinase K Solution to each sample.**
4. **Vortex for 60 seconds to mix thoroughly.**
5. **Incubate at 65°C for 30 minutes. Mix once during incubation.**
6. **Centrifuge at 3,500 x g for 10 minutes.**
7. **Transfer 300 µL cleared supernatant to a 1.5mL Tube**
8. **Add 300 µL PBG Buffer, 300 µL PBB Buffer, and 20 µL Mag-RQ1 Particles to each sample. Vortex to mix thoroughly or pipet up and down 20 times.**

   ⚠ Shake thoroughly the MAG-Bind particles to fully resuspend before use.
9. **Let sit at room temperature for 10 minutes.**

10. **Place the 1.5mL tube on the Magnetic Separation Device to magnetize the Mag-RQ1 Particles. Let sit at room temperature until the Mag-RQ1 Particles are completely cleared from solution.**

11. **Aspirate and discard the cleared supernatant. Do not disturb the Mag-RQ1 Particles.**

12. **Remove the tube containing the Mag-RQ1 Particles from the Magnetic Separation Device.**

13. **Add 600 µL PBH Buffer to each sample. Resuspend the Mag-RQ1 Particles by vortexing or pipetting up and down 20 times.**
    **Note: PBH Buffer must be diluted with 100% ethanol prior to use. Please see Page 2 for instructions.**

14. **Let sit at room temperature for 2 minutes.**

15. **Place the 1.5mL tube on the Magnetic Separation Device to magnetize the Mag-RQ1 Particles. Let sit at room temperature until the Mag-RQ1 Particles are completely cleared from solution.**

16. **Aspirate and discard the cleared supernatant. Do not disturb the Mag-RQ1 Particles.**

17. **Remove the tube containing the Mag-RQ1 Particles from the Magnetic Separation Device.**

18. **Repeat Steps 13-17 once for a second VHB Wash step.**

19. **Add 600 µL MQ Wash Buffer to each sample. Resuspend the Mag-RQ1 Particles by vortexing or pipetting up and down 20 times.**
    **Note: MQ Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 2 for instructions.**

20. **Let sit at room temperature for 2 minutes.**

21. **Place the 1.5mL tube on the Magnetic Separation Device to magnetize the Mag-RQ1 Particles. Let sit at room temperature until the Mag-RQ1 Particles are completely cleared from solution.**

22. **Aspirate and discard the cleared supernatant. Do not disturb the Mag-RQ1 Particles.**

23. **Leave the tube on the Magnetic Separation Device.**

24. **Add 500 µL nuclease-free water (not provided) to each sample. Immediately aspirate the nuclease free water. Do not let the samples stay in contact with the nuclease-free water for more than 60 seconds.**
    **Note: If using an automated platform, use the maximum volume the tips will allow up to 600 µL.**

25. **Add 50-100 µL MB Elution Buffer heated to 70°C to each sample. Resuspend the Mag-RQ1 Particles by vortexing or pipetting up and down 20 times.**

26. **Let sit at room temperature for 5 minutes.**

27. **Place the 1.5mL tube on the Magnetic Separation Device to magnetize the Mag-RQ1 Particles. Let sit at room temperature until the Mag-RQ1 Particles are completely cleared**

**from solution.**

**28. Transfer the cleared supernatant containing purified DNA to a clean 1.5mL tube. Store the DNA at -20°C.**

# HighPrep™ Pathogen Maxi Kit : Serum and Stool Protocol

## Materials and Equipment to be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ Centrifuge capable of at least 3,500 x g
- ☐ Magnetic Separation Device (MBMS-10)
- ☐ Incubator capable of 65°C
- ☐ 1.5ml tubes
- ☐ Vortexer
- ☐ 100% ethanol
- ☐ Nuclease Free Water

## Things to do before starting

- ☐ Prepare PBH Buffer and MQ Wash Buffer according to the "Preparing Reagents" section on Page 2.
- ☐ Set an incubator to 70°C.
- ☐ Heat Elution Buffer to 70°C

## Protocol

1. **Add 250 µL serum or stool samples to each 1.5mL tube. If stool sample is solid, resuspend to 10% wt/volume in PBS before starting.**
2. **Add 275 µL PL Buffer to each sample.**
3. **Add 50µL PB Buffer and 20 µL Proteinase K Solution to each sample.**
4. **Vortex for 60 seconds to mix thoroughly.**
5. **Incubate at 65°C for 30 minutes. Mix once during incubation.**
6. **Centrifuge at 3,500 x g for 10 minutes.**
7. **Transfer 300 µL cleared supernatant to a 1.5mL Tube.**
8. **Add 300 µL PBG Buffer, 300 µL PBB Buffer, and 20 µL Mag-RQ1 Particles to each sample. Vortex to mix thoroughly or pipet up and down 20 times.**

   ⚠ Shake thoroughly the MAG-Bind particles to fully resuspend before use.
9. **Let sit at room temperature for 10 minutes.**
10. **Place the 1.5mL tube on the Magnetic Separation Device to magnetize the Mag-RQ1**

**Particles. Let sit at room temperature until the Mag-RQ1 Particles are completely cleared from solution.**

11. **Aspirate and discard the cleared supernatant. Do not disturb the Mag-RQ1 Particles.**

12. **Remove the tube containing the Mag-RQ1 Particles from the Magnetic Separation Device.**

13. **Add 600 µL PBH Buffer to each sample. Resuspend the Mag-RQ1 Particles by vortexing or pipetting up and down 20 times.**
    **Note: PBH Buffer must be diluted with 100% ethanol prior to use. Please see Page 2 for instructions.**

14. **Let sit at room temperature for 2 minutes.**

15. **Place the 1.5mL tube on the Magnetic Separation Device to magnetize the Mag-RQ1 Particles. Let sit at room temperature until the Mag-RQ1 Particles are completely cleared from solution.**

16. **Aspirate and discard the cleared supernatant. Do not disturb the Mag-RQ1 Particles.**

17. **Remove the tube containing the Mag-RQ1 Particles from the Magnetic Separation Device.**

18. **Repeat Steps 13-17 once for a second PBH Wash step.**

19. **Add 600 µL MQ Wash Buffer to each sample. Resuspend the Mag-RQ1 Particles by vortexing or pipetting up and down 20 times.**
    **Note: MQ Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 2 for instructions.**

20. **Let sit at room temperature for 2 minutes.**

21. **Place the 1.5mL tube on the Magnetic Separation Device to magnetize the Mag-RQ1 Particles. Let sit at room temperature until the Mag-RQ1 Particles are completely cleared from solution.**

22. **Aspirate and discard the cleared supernatant. Do not disturb the Mag-RQ1 Particles.**

23. **Leave the tube on the Magnetic Separation Device.**

24. **Add 500 µL nuclease-free water (not provided) to each sample. Immediately aspirate the nuclease free water. Do not let the samples stay in contact with the nuclease-free water for more than 60 seconds.**
    **Note: If using an automated platform, use the maximum volume the tips will allow up to 600 µL.**

25. **Add 50-100 µL MB Elution Buffer heated to 70°C to each sample. Resuspend the Mag-RQ1 Particles by vortexing or pipetting up and down 20 times.**

26. **Let sit at room temperature for 5 minutes.**

27. **Place the 1.5mL tube on the Magnetic Separation Device to magnetize the Mag-RQ1**

**Particles. Let sit at room temperature until the Mag-RQ1 Particles are completely cleared from solution.**

**28. Transfer the cleared supernatant containing purified DNA to a clean1.5mL tube. Store the DNA at -20°C.**

# HighPrep™ Pathogen Maxi Kit : Urine Protocol

## Materials and Equipment to be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ Centrifuge capable of at least 3,500 x g
- ☐ Magnetic Separation Device (MBMS-10)
- ☐ Incubator capable of 65°C
- ☐ 1.5ml tubes
- ☐ Vortexer
- ☐ 100% ethanol
- ☐ Nuclease Free Water

## Things to do before starting

- ☐ Prepare PBH Buffer and MQ Wash Buffer according to the "Preparing Reagents" section on Page 2.
- ☐ Set an incubator to 70°C.
- ☐ Heat Elution Buffer to 70°C
- ☐ Prepare an Ice bucket

## Protocol

1. **Add 250 µL urine sample to each 1.5mL tube.**
2. **Add 275 µL PL Buffer to each sample.**
3. **Add 50µL PB Buffer and 20 µL Proteinase K Solution to each sample.**
4. **Vortex for 60 seconds to mix thoroughly.**
5. **Incubate at 65°C for 30 minutes. Mix once during incubation.**
6. **Add 200 µL PP Buffer to each well. Place the plate on ice for 5 minutes**
7. **Centrifuge at 3,500 x g for 10 minutes.**
8. **Transfer 300 µL cleared supernatant to a 1.5mL tube.**
9. **Add 300 µL PBG Buffer, 300 µL RBB Buffer, and 20 µL Mag-RQ1 Particles to each sample. Vortex to mix thoroughly or pipet up and down 20 times.**

   ⚠ Shake thoroughly the MAG-Bind particles to fully resuspend before use.
10. **Let sit at room temperature for 10 minutes.**

**11. Place the 1.5mL tube on the Magnetic Separation Device to magnetize the Mag-RQ1 Particles. Let sit at room temperature until the Mag-RQ1 Particles are completely cleared from solution.**

**12. Aspirate and discard the cleared supernatant. Do not disturb the Mag-RQ1 Particles.**

**13. Remove the tube containing the Mag-RQ1 Particles from the Magnetic Separation Device.**

**14. Add 600 µL PBH Buffer to each sample. Resuspend the Mag-RQ1 Particles by vortexing or pipetting up and down 20 times.**
**Note: PBH Buffer must be diluted with 100% ethanol prior to use. Please see Page 2 for instructions.**

**15. Let sit at room temperature for 2 minutes.**

**16. Place the 1.5mL tube on the Magnetic Separation Device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-RQ1 Particles are completely cleared from solution.**

**17. Aspirate and discard the cleared supernatant. Do not disturb the Mag-RQ1 Particles.**

**18. Remove the tube containing the Mag-RQ1 Particles from the Magnetic Separation Device.**

**19. Repeat Steps 13-17 once for a second PBH Wash step.**

**20. Add 600 µL MQ Wash Buffer to each sample. Resuspend the Mag-RQ1 Particles by vortexing or pipetting up and down 20 times.**
**Note: MQ Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 2 for instructions.**

**21. Let sit at room temperature for 2 minutes.**

**22. Place the 1.5mL tube on the Magnetic Separation Device to magnetize the Mag-RQ1 Particles. Let sit at room temperature until the Mag-RQ1 Particles are completely cleared from solution.**

**23. Aspirate and discard the cleared supernatant. Do not disturb the Mag-RQ1 Particles.**

**24. Leave the tube on the Magnetic Separation Device.**

**25. Add 500 µL nuclease-free water (not provided) to each sample. Immediately aspirate the nuclease free water. Do not let the samples stay in contact with the nuclease-free water for more than 60 seconds.**
**Note: If using an automated platform, use the maximum volume the tips will allow up to 600 µL.**

**26. Add 50-100 µL MB Elution Buffer heated to 70°C to each sample. Resuspend the Mag-RQ1 Particles by vortexing or pipetting up and down 20 times.**

**27. Let sit at room temperature for 5 minutes.**

**28. Place the 1.5mL tube on the Magnetic Separation Device to magnetize the Mag-RQ1 Particles. Let sit at room temperature until the Mag-RQ1 Particles are completely cleared from solution.**

**29. Transfer the cleared supernatant containing purified DNA to a clean 1.5mL tube. Store the DNA at -20°C.**

# Troubleshooting guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:
Phone: 1-855-262-4246 (in US), outside US, 1-301-302-0144
Email: support@magbiogenomics.com

| Symptoms | Possible Causes | Comments |
|---|---|---|
| A260/A230 ratio is low | Salt contamination | • Repeat the DNA isolation with a new sample.<br>• Extend the incubation time with VHB Buffer.<br>• Wash the Mag-Bind® Particles RQ with ethanol. |
| A260/A280 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 Wash Buffer are mixed with ethanol. |
| | Water Wash extended | 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. Let magnetic beads remagnetize prior to aspirating liquid. |
| 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 elute used in the downstream application if possible. |
| | Non-specific bands in downstream PCR | Use hot-start Taq polymerase mixture |
| | Inhibitory substance in the eluted DNA. | Check the A260/A230 ratio.<br>Dilute the elute to 1:50 if necessary. |

# Ordering and Related Product Information

### Post PCR and Next Gen library prep clean up system

| Catalog No. | Product |
|---|---|
| AC-60005 | HighPrep PCR (5 mL) |
| AC-60050 | HighPrep PCR (50 mL) |
| AC-60500 | HighPrep PCR (500 mL) |

### BigDye Sanger Sequencing Cleanup

| Catalog No. | Product |
|---|---|
| DT-70005 | HighPrep DTR (5 mL) |
| DT-70050 | HighPrep DTR (50 mL) |
| DT-70500 | HighPrep DTR (500 mL) |

### Magnetic Separation Devices

| Catalog No. | Description |
|---|---|
| MYMAG-96 | Handheld Magnetic Separation Device (96 well microplate format) |
| MBMS-10 | MagStip magnetic stand (1.5 mL x 10) |
| MBMS-31550 | 15ml and 50ml magnetic stand combo. (3x15ml and 3x50ml) |

### cfDNA Purification Kit

| Catalog No. | Product | Description | Preps |
|---|---|---|---|
| CFK-D10-400UL | CF-Kapture 21 Kit (200-400µl) 10 preps | Purification of cell-free DNA (cfDNA) from 200-400 µl STABILIZED plasma | 10 |
| CFK-D5-5ML | CF-Kapture 21 Kit (3-5ml ) 5 preps | Purification of cell-free DNA (cfDNA) from 3-5 ml STABILIZED plasma | 5 |
| CFK-D50-400UL | CF-Kapture 21 Kit (200-400µl )(50 preps) | Purification of cell-free DNA (cfDNA) from 200-400 µl STABILIZED plasma | 50 |
| CFK-D50-2ML | CF-Kapture 21 Kit (1-2ml) 50 preps | Purification of cell-free DNA (cfDNA) from 1-2 ml STABILIZED plasma | 50 |
| CFK-D50-5ML | CF-Kapture 21 Kit (3-5 ml) 50 preps | Purification of cell-free DNA (cfDNA) from 3-5 ml STABILIZED plasma | 50 |

### Whole blood stabilization tubes

| Catalog No. | Product | Description |
|---|---|---|
| BS21-CF10-100 | Blood STASIS 21-ccfDNA 9 mL (100) | 100 tubes: 2 ml Additive, 7 ml blood draw volume |
| BS21-CF6-100 | Blood STASIS 21-ccfDNA 6 mL (100) | 100 tubes: 1.5 ml Additive, 4.5 ml blood draw volume |
| BS21-CF3-200 | Blood STASIS 21-ccfDNA 3 mL (200) | 200 tubes: 0.5 ml Additive, 2.5 ml blood draw volume |

**RNA or cDNA for in vitro applications clean up system**

| Catalog No. | Product |
|---|---|
| RC-60005 | HighPrep RNA Elite (5 mL) |
| RC-60050 | HighPrep RNA Elite (50 mL) |
| RC-60500 | HighPrep RNA Elite (500 mL) |


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