Clean Pathogen DNA / RNA Kit

Catalog Nos. CPT-DR0096, CPT-DR0384
Manual revision v4.00

Contents

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Introduction and Principle

The Clean Pathogen Kit is designed for high throughput and reliable isolation from tissue, urine, whole blood, serum, and fecal samples of both DNA and RNA during each isolation.
The Clean Pathogen Kit will isolate DNA from:

- high quality host genomic DNA
- gram positive and negative bacterial DNA
- fungal spore DNA
- viral DNA

The Clean Pathogen Kit will isolate RNA from:

- host RNA
- viral RNA

The kits protocol is fully scalable and due to the use of our magnetic bead purification technology, can besides manual usage, easily be automated once the samples have been lysed on liquid handling workstations (e.g. Dynamic Devices LYNX™, Hamilton STAR™, Thermo KingFisher™ Flex, Applied Biosystems® MagMAX™, Qiagen BioSprint, and other liquid handling instruments).

The system combines the CleanNA technology with our specially formulated buffer system to eliminate the binding of PCR inhibiting compounds, present within the samples, onto our magnetic particles. There are no organic extractions thus reducing plastic waste and hands-on time and multiple samples can be processed in parallel.

Purified DNA is suitable for PCR, qPCR, restriction digestion, Next Generation Sequencing and hybridization applications.
Purified RNA is suitable for reverse transcription for cDNA synthesis, RT-PCR, RT-qPCR and RNA-Seq.

Using our specially formulated lysis buffer, samples are lysed and the DNA and RNA is bound to our magnetic particles while DNases and RNases are deactivated. DNA and RNA is isolated from the lysates in one step by binding to the CleanNA Particles' surfaces. The CleanNA magnetic particles are separated from the lysates by using a magnetic separation device. Following a few rapid wash steps to remove trace contaminants, the purified DNA/RNA is eluted from the CleanNA particles for downstream applications using an Elution Buffer.
For downstream DNA applications, we advise to store the eluted DNA at -20°C.
For downstream DNA and/or RNA applications, we advise to store the eluted DNA/RNA at -80°C.

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Kit Contents and Materials

Kit Contents:

Product	CPT-D0096	CPT-D0384	Storage
Preps	1 x 96	4 x 96	
Lysis Buffer CPT	60 mL	240 mL	15-25^{0}C
Clean Disruptor Plate	1	4	
Caps	13	52	
PK Buffer CPT	8 mL	30 mL	15-25^{0}C
Proteinase K Solution	2.2 mL	9 mL	15-25°C (for storage > 12 months, store at 2-8°C)
Binding Buffer CPT	40 mL	160 mL	15-25^{0}C
CPT Prep Buffer	40 mL	160 mL	15-25^{0}C
CleanNA Particles CPT	2.2 mL	9 mL	2-8^{0}C
CPT Wash Buffer 1	88 mL	3 x 88 mL	15-25^{0}C
CPT Wash Buffer 2	30 mL	4 x 30 mL	15-25^{0}C
Elution Buffer	15 mL	50 mL	15-25^{0}C
CPT+ Reagent	25 mL	100 mL	15-25^{0}C

Check all buffers for precipitates prior to usage. Any precipitates can be re-dissolved by warming the buffer(s) to 37^{0}C.

Materials and Reagents to be supplied by User for Tissue and Serum & Stool protocol:

- Centrifuge capable of at least 3,500 x g with adaptor for 96-well plates
- Magnetic separation device for 96-well plates (CleanNA, Part# CMAG-RN50))
- 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 Qiagen TissueLyser

Materials and Reagents to be supplied by User for Urine & Whole Blood protocol:

- Centrifuge capable of at least 3,500 x g with adaptor for 96-well plates
- Magnetic separation device for 96-well plates (CleanNA, Part# CMAG-RN50)
- 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
- For processing Whole Blood: 2 mL screw cap tubes
- Vortexer
- 100% ethanol
- Nuclease-free water
- Optional: Mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser
- Ice Bucket

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Preparation of Reagents

Prepare CPT Wash Buffer 1 with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
CPT-DR0096	112 mL
CPT-DR0384	112 mL per bottle

Dilute CPT Wash Buffer 2 with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
CPT-DR0096	70 mL
CPT-DR0384	70 mL per bottle

Shake or vortex the CleanNA Particles CPT to fully resuspend the particles prior to usage. The particles must be fully suspended during use to ensure proper binding.

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Clean Pathogen Kit
Tissue Protocol

Before Starting:

- Prepare CPT Wash Buffer 1 and CPT Wash Buffer 2 according to the “Preparation of Reagents” section on Page 4.
- Set an incubator to 70°C
- Heat Elution Buffer to 70°C

Protocol:

1. Briefly spin the Clean Disruptor Plate to remove any glass beads from the walls of the wells. Uncap the Clean Disruptor Plate and save the caps for use in Step 3.

2. Add 25-30 mg tissue to each well.

3. Add 525 µL Lysis Buffer CPT to each sample. Seal the Clean Disruptor Plate 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 Qiagen Tissuelyser, should be used.

   

   Note: Depending on the sample amount and type, the amount of Lysis Buffer CPT may need to be adjusted so that 300 µL can be recovered after Step 11.

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

6. Remove and discard the caps from the Clean Disruptor Plate.

7. Add 53 µL PK Buffer CPT and 20 µL Proteinase K Solution to each sample.

8. Seal the Clean Disruptor Plate with new Caps for Racked Microtubes (provided).

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 x 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 Binding Buffer CPT, 300 µL CPT Prep Buffer, and 20 µL CleanNA Particles CPT to each sample. Vortex to mix thoroughly or pipet up and down 20 times.

    

    Note: CleanNA Particles CPT and Binding Buffer CPT can be prepared as a master mix prior to use. Prepare only what is needed. Tip mixing is recommended for automated protocols for best yield.

14. Incubate at room temperature for 10 minutes.

15. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

16. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

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17. Remove the plate containing the CleanNA Particles CPT from the Magnetic Separation Device.

18. Add 600 µL CPT Wash Buffer 1 to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

    Note: CPT Wash Buffer 1 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

19. Incubate at room temperature for 2 minutes.

20. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

21. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

22. Remove the plate containing the CleanNA Particles CPT from the Magnetic Separation Device.

23. Repeat Steps 18-22 once for a second CPT Wash Buffer 1 step.

24. Add 600 µL CPT Wash Buffer 2 to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

    Note: CPT Wash Buffer 2 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

25. Incubate at room temperature for 2 minutes.

26. Place the 96-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

27. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

28. Leave the plate on the Magnetic Separation Device.

29. Add 500 µL nuclease-free water (not provided) to each sample. Immediately aspirate and remove the nuclease free water. Do not let the samples stay in contact with the nuclease-free water for more than 60 seconds.

    Note: This step (water "wash") is crucial to remove traces of ethanol from the sample. When adding water, the CleanNA Particles CC will come out of the ring shape shortly. This is a normal phenomenon, the CleanNA particles CC will return to the ring shape after a few seconds.

    Note: 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 to each sample. Resuspend CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

31. Incubate at room temperature for 5 minutes.

32. Place the 96-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

33. Transfer the cleared supernatant containing purified DNA/RNA to a clean 96-well microplate. Store the DNA at -20°C. For downstream RNA applications, store the eluate at -80°C.

Clean Pathogen Kit
Serum & Stool Protocol

Before Starting:

- Prepare CPT Wash Buffer 1 and CPT Wash Buffer 2 according to the “Preparation of Reagents” section on Page 5.
- Set an incubator to 70°C
- Heat Elution Buffer to 70°C

Protocol:

1. Briefly spin the Clean Disruptor Plate to remove any glass beads from the walls of the wells. Uncap the Clean Disruptor Plate and save the caps for use in Step 3.

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

3. Add 275 µL Lysis Buffer CPT to each sample. Seal the Clean Disruptor Plate 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 Qiagen Tissuelyser, should be used.

   ⚠ **Note:** Depending on the sample amount and type, the amount of Lysis Buffer CPT may need to be adjusted so that 300 µL can be recovered after Step 11.

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

6. Remove and discard the caps from the Clean Disruptor Plate.

7. Add 50µL PK Buffer CPT and 20 µL Proteinase K Solution to each sample.

8. Seal the Clean Disruptor Plate with new Caps for Racked Microtubes (provided).

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 x 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 Binding Buffer CPT, 300 µL CPT Prep Buffer, and 20 µL CleanNA Particles CPT to each sample. Vortex to mix thoroughly or pipet up and down 20 times.

    ⚠ **Note:** CleanNA Particles CPT and Binding Buffer CPT can be prepared as a master mix prior to use. Prepare only what is needed. Tip mixing is recommended for automated protocols for best yield.

14. Incubate at room temperature for 10 minutes.

15. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

16. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

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17. Remove the plate containing the CleanNA Particles CPT from the Magnetic Separation Device.

18. Add 600 µL CPT Wash Buffer 1 to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

    Note: CPT Wash Buffer 1 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

19. Incubate at room temperature for 2 minutes.

20. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

21. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

22. Remove the plate containing the CleanNA Particles CPT from the Magnetic Separation Device.

23. Repeat Steps 18-22 once for a second CPT Wash Buffer 1 step.

24. Add 600 µL CPT Wash Buffer 2 to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

    Note: CPT Wash Buffer 2 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

25. Incubate at room temperature for 2 minutes.

26. Place the 96-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

27. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

28. Leave the plate on the Magnetic Separation Device.

29. Add 500 µL nuclease-free water (not provided) to each sample. Immediately aspirate and remove the nuclease free water. Do not let the samples stay in contact with the nuclease-free water for more than 60 seconds.

    Note: This step (water "wash") is crucial to remove traces of ethanol from the sample. When adding water, the CleanNA Particles CC will come out of the ring shape shortly. This is a normal phenomenon, the CleanNA particles CC will return to the ring shape after a few seconds.

    Note: 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 to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

31. Incubate at room temperature for 5 minutes.

32. Place the 96-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

33. Transfer the cleared supernatant containing purified DNA/RNA to a clean 96-well microplate. Store the DNA at -20°C. For downstream RNA applications, store the eluate at -80°C.

Clean Pathogen Kit
Urine and Whole Blood Protocol

Before Starting:

- Prepare CPT Wash Buffer 1 and CPT Wash Buffer 2 according to the “Preparation of Reagents” section on Page 5.
- Set an incubator to 70°C
- Heat Elution Buffer to 70°C
- Prepare an ice Bucket

Protocol:

1. Briefly spin the Clean Disruptor Plate to remove any glass beads from the walls of the wells. Uncap the Clean Disruptor Plate and save the caps for use in Step 3.

2. Sample addition:

    a. For urine, add 250 µL urine samples to each well of the Clean Disruptor plate
    b. For Whole Blood, transfer 250 µL sample into a clean screw cap tube (not provided). To each screw cap tube, add the content of 1 Clean Disruptor Plate tube, ensuring all grinding beads are added to the tube..

    

    Note: Whole Blood needs to be processed in a screw cap tube in order to prevent cross contamination, since it will foam quite intensively during homogenization.

3. Add 275 µL Lysis Buffer CPT to each sample. Seal the Clean Disruptor Plate (for urine) with the caps removed in Step 1. For Whole Blood close the screw cap tube, using its screw cap.

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 Qiagen Tissuelyser, should be used.

    

    Note: Depending on the sample amount and type, the amount of Lysis Buffer CPT may need to be adjusted so that 300 µL can be recovered after Step 12.

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

6. Remove and discard the caps from the Clean Disruptor Plate or the screw cap tubes.

7. Add 50µL PK Buffer CPT and 20 µL Proteinase K Solution to each sample.

8. Seal the Clean Disruptor Plate with new Caps for Racked Microtubes (provided) or re-use the screw cap from the screw cap tube.

9. Vortex for 60 seconds to mix thoroughly.

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

11. Add 200 µL CPT+ Reagent to each well. Place the plate on ice for 5 minutes.

12. Centrifuge at 3,500 x 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 yield and purity.

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14. Add 300 µL Binding Buffer CPT, 300 µL CPT Prep Buffer, and 20 µL CleanNA Particles CPT to each sample. Vortex to mix thoroughly or pipet up and down 20 times.

    Note: CleanNA Particles CPT and Binding Buffer CPT can be prepared as a master mix prior to use. Prepare only what is needed. Tip mixing is recommended for automated protocols for best yield.

15. Incubate at room temperature for 10 minutes.

16. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.
17. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

18. Remove the plate containing the CleanNA Particles CPT from the Magnetic Separation Device.

19. Add 600 µL CPT Wash Buffer 1 to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

    Note: CPT Wash Buffer 1 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

20. Incubate at room temperature for 2 minutes.

21. Place the 96-well deep-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

22. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

23. Remove the plate containing the CleanNA Particles CPT from the Magnetic Separation Device.

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

25. Add 600 µL CPT Wash Buffer 2 to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

    Note: CPT Wash Buffer 2 must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

26. Incubate at room temperature for 2 minutes.

27. Place the 96-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

28. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CPT.

29. Leave the plate on the Magnetic Separation Device.

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

    Note: This step (water “wash”) is crucial to remove traces of ethanol from the sample. When adding water, the CleanNA Particles CC will come out of the ring shape shortly. This is a normal phenomenon, the CleanNA particles CC will return to the ring shape after a few seconds.

    Note: If using an automated platform, use the maximum volume the tips will allow up to 600 µL.

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31. Add 50-100 µL Elution Buffer heated to 70°C to each sample. Resuspend the CleanNA Particles CPT by vortexing or pipetting up and down 20 times.

32. Incubate at room temperature for 5 minutes.

33. Place the 96-well plate on the Magnetic Separation Device to magnetize the CleanNA Particles CPT. Incubate at room temperature until the CleanNA Particles CPT are completely cleared from solution.

34. Transfer the cleared supernatant containing purified DNA/RNA to a clean 96-well microplate. Store the DNA at -20°C. For downstream RNA applications, store the eluate at -80°C..

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Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact your local distributor.

Possible Problems and Suggestions

Problem	Cause	Solution
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 Lysis Buffer CPT thoroughly. Use a commercial homogenizer if possible.
	Incomplete Resuspension of CleanNA Particles CPT	Resuspend the CleanNA Particles CPT by vortexing vigorously before use
	DNA washed off	Make sure CPT Wash Buffer 1 and CPT Wash Buffer 2 are mixed with ethanol.
		Ensure the water “wash” does not exceed 60 seconds and the CleanNA Particles CPT are not resuspended.
	CleanNA Particles CPT lost in process	After water is added during wash step CleanNA Particles CPT will go into solution. Let magnetic beads re-magnetize prior to aspirating liquid.
cDNA synthesis not working	Degraded RNA	Store the eluate at -80°C after isolation and minimize the number of freeze thaw cycles.
		Ensure to work RNase free and use RNase free materials during the isolation process and in downstream applications
CleanNA Particles CPT do not completely clear from solution	Too short of magnetizing time	Increase the particle collection time on the magnetic separation device
A260/A230 ratio is low	Salt contamination	• Repeat the DNA isolation with a new sample. • Extend the incubation time with CPT Wash Buffer 1. • Wash the CleanNA Particles CPT 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. Incubate at room temperature for 5 minutes.

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Problem	Cause	Solution
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 A_{260}/A_{230} ratio. Dilute the elute to 1:50 if necessary.
Abnormal BioAnalyzer data	BioAnalyzer shows multiple sharp peaks during the analysis	Ensure to remove all traces of the cleared supernatant after each wash step
		Ensure to perform a water “wash” to remove final ethanol traces
	BioAnalyzer shows base line climbing towards the end	Check the BioAnalyzer chip for air bubbles. Load samples onto a new freshly prepared chip
	BioAnalyzer shows high blob at the beginning of the trace	Ensure the purified sample does not contain traces of CleanNA Particles CC

Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean Pathogen DNA / RNA Kit 96 Preps	CPT-DR0096
Clean Pathogen DNA / RNA Kit 384 Preps	CPT-DR0384

Product	Part Number
Clean Magnet Plate 96-Well	CMAG-96-RN50

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